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=> s B cell epitope determination
   3 FILES SEARCHED...
L1            0 B CELL EPITOPE DETERMINATION

=> s method
L2     10218462 METHOD

=> s l2 and allergen
L3        25207 L2 AND ALLERGEN

=> s l3 and B cell
   3 FILES SEARCHED...
L4          583 L3 AND B CELL

=> s l4 and epitope determination
L5            0 L4 AND EPITOPE DETERMINATION

=> s l4 and epitope
L6          256 L4 AND EPITOPE

=> s l6 and angstrom
L7            0 L6 AND ANGSTROM

=> s l6 and surface exposure
L8            0 L6 AND SURFACE EXPOSURE

=> s l6 and amino acid substitution
L9           10 L6 AND AMINO ACID SUBSTITUTION

=> dup remove l9
PROCESSING COMPLETED FOR L9
L10           5 DUP REMOVE L9 (5 DUPLICATES REMOVED)

=> s l10 and IgE binding
L11           2 L10 AND IGE BINDING

=> dup remove l11
PROCESSING COMPLETED FOR L11
L12           2 DUP REMOVE L11 (0 DUPLICATES REMOVED)

=> d l12 1-2 cbib abs

L12  ANSWER 1 OF 2  SCISEARCH  COPYRIGHT 2002 ISI (R)
2000:153581   The Genuine Article (R) Number: 285RQ. Mutational analysis of the
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IgE-binding epitopes of P34/Gly m Bd 30K.
Helm R M (Reprint); Cockrell G; Connaughton C; West C M; Herman E; Sampson H A; Bannon G A; Burks A W. UNIV ARKANSAS MED SCI, ARKANSAS CHILDRENS NUTR CTR, DEPT PEDIAT, 1120 MARSHALL ST, LITTLE ROCK, AR 72202 (Reprint); UNIV ARKANSAS MED SCI, DEPT BIOCHEM & MOL BIOL, LITTLE ROCK, AR 72202; AGR RES SERV, CLIMATE STRESS LAB, USDA, BELTSVILLE, MD; MT SINAI SCH MED, DIV PEDIAT ALLERGY & IMMUNOL, NEW YORK, NY. JOURNAL OF ALLERGY AND CLINICAL IMMUNOLOGY (FEB 2000) Vol. 105, No. 2, Part 1, pp. 378-384. Publisher: MOSBY-YEAR BOOK INC. 11830 WESTLINE INDUSTRIAL DR, ST LOUIS, MO 63146-3318 . ISSN: 0091-6749. Pub. country: USA. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Background: Peanuts and soybeans are 2 foods that have been shown to be responsible for many atopic disorders. Because of their nutritional benefit, soybean proteins are now being used increasingly in a number of food products. Previous studies have documented multiple **allergens** in soybean extracts, including glycinin, beta-conglycinin, and the P34/Gly m Bd 30K protein.

Objective: Our overall goal was to identify soybean-specific **allergens** to begin to understand molecular and immunochemical characteristics of legume proteins. The specific aim of the current investigation was to identify the essential amino acid residues necessary for **IgE binding** in the 5 distinct immunodominant **epitopes** of P34/Gly m Ed 30K,

Methods: Serum IgE from 6 clinically sensitive soybean-allergic individuals was used to identify P34/Gly m Ed 30K in the native and single amino acid substituted peptides with use of the SPOTS peptide synthesis technique to determine critical amino acids required for **IgE binding**.

Results: The intensity of **IgE binding** and **epitope** recognition by serum IgE from the individuals varied substantially. With use of serum from 6 clinically soybean-sensitive individuals, 2 of the 5 immunodominant **epitopes** could be mutagenized to non-**IgE binding** peptides,

Conclusions: Single-site **amino acid substitution** of the 5 immunodominant **epitopes** of Gly m Ed 30K with alanine revealed that **IgE binding** could be reduced or eliminated in **epitopes** 6 and 16 in the serum obtained from 6 soybean-sensitive patients.

L12 ANSWER 2 OF 2 MEDLINE
2001086248 Document Number: 20564013. PubMed ID: 11112857. A soybean G2 glycinin **allergen**. 2. **Epitope** mapping and three-dimensional modeling. Helm R M; Cockrell G; Connaughton C; Sampson H A; Bannon G A; Beilinson V; Nielsen N C; Burks A W. (Department of Pediatrics, University of Arkansas for Medical Sciences, Arkansas Children's Nutrition Center, Little Rock, AR 72202-3591, USA.) INTERNATIONAL ARCHIVES OF ALLERGY AND IMMUNOLOGY, (2000 Nov) 123 (3) 213-9. Journal code: 9211652. ISSN: 1018-2438. Pub. country: Switzerland. Language: English.

AB BACKGROUND: Multiple **allergens** have been documented in soybean extracts. IgE from individuals allergic to soybeans, but not to peanut, has been shown by immunoblot analysis to bind to proteins with a molecular weight of approximately 22 kD. These findings suggested that this unique protein fraction from soybean might be responsible, in part, for soybean allergic reactivity. The objective of the present study was to characterize specific **B cell epitopes**, to determine if any amino acid was critical to **IgE binding** and to model the 22-kD G2 soybean **allergen** to the three-dimensional (3-D) phaseolin molecule. **METHODS**: **B cell epitopes** were identified using SPOTs peptide analysis. Structural orientation of the **IgE-binding** regions was mapped to the 3-D phaseolin molecule using molecular modeling of the protein tertiary structure. RESULTS: Eleven linear **epitopes**

, representing 15 amino acid peptide sequences, bound to IgE in the glycinin molecule. These **epitopes** were predicted to be distributed asymmetrically on the surface of G2 trimers. CONCLUSIONS: Only 1 **epitope** could be rendered non-**IgE binding** by alanine substitutions in the peptide. The nonrandom distribution of the **IgE binding** sites provides new insight into their organization in trimers in 11S complexes of the G2 glycinin **allergen**.

=> d l10 1-5 cbib abs

L10 ANSWER 1 OF 5 SCISEARCH COPYRIGHT 2002 ISI (R)
2000:153581 The Genuine Article (R) Number: 285RQ. Mutational analysis of the IgE-binding **epitopes** of P34/Gly m Bd 30K. Helm R M (Reprint); Cockrell G; Connaughton C; West C M; Herman E; Sampson H A; Bannon G A; Burks A W. UNIV ARKANSAS MED SCI, ARKANSAS CHILDRENS NUTR CTR, DEPT PEDIAT, 1120 MARSHALL ST, LITTLE ROCK, AR 72202 (Reprint); UNIV ARKANSAS MED SCI, DEPT BIOCHEM & MOL BIOL, LITTLE ROCK, AR 72202; AGR RES SERV, CLIMATE STRESS LAB, USDA, BELTSVILLE, MD; MT SINAI SCH MED, DIV PEDIAT ALLERGY & IMMUNOL, NEW YORK, NY. JOURNAL OF ALLERGY AND CLINICAL IMMUNOLOGY (FEB 2000) Vol. 105, No. 2, Part 1, pp. 378-384. Publisher: MOSBY-YEAR BOOK INC. 11830 WESTLINE INDUSTRIAL DR, ST LOUIS, MO 63146-3318 . ISSN: 0091-6749. Pub. country: USA. Language: English.
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Conclusions: Single-site **amino acid substitution** of the 5 immunodominant **epitopes** of Gly m Ed 30K with alanine revealed that IgE binding could be reduced or eliminated in **epitopes** 6 and 16 in the serum obtained from 6 soybean-sensitive patients.

L10 ANSWER 2 OF 5 MEDLINE
2001086248 Document Number: 20564013. PubMed ID: 11112857. A soybean G2 glycinin **allergen**. 2. **Epitope** mapping and three-dimensional modeling. Helm R M; Cockrell G; Connaughton C; Sampson H A; Bannon G A; Beilinson V; Nielsen N C; Burks A W. (Department of Pediatrics, University of Arkansas for Medical Sciences, Arkansas Children's Nutrition Center, Little Rock, AR 72202-3591, USA.) INTERNATIONAL ARCHIVES OF ALLERGY AND IMMUNOLOGY, (2000 Nov) 123 (3) 213-9. Journal code: 9211652. ISSN: 1018-2438. Pub. country: Switzerland. Language: English.

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L10 ANSWER 3 OF 5 MEDLINE DUPLICATE 1
1998224476 Document Number: 98224476. PubMed ID: 9564806. Antagonistic peptides specifically inhibit proliferation, cytokine production, CD40L expression, and help for IgE synthesis by Der p 1-specific human T-cell clones. Fasler S; Aversa G; de Vries J E; Yssel H. (Human Immunology Department, DNAX Research Institute for Molecular and Cellular Biology, Palo Alto, Calif, USA.) JOURNAL OF ALLERGY AND CLINICAL IMMUNOLOGY, (1998 Apr) 101 (4 Pt 1) 521-30. Journal code: 1275002. ISSN: 0091-6749. Pub. country: United States. Language: English.

AB BACKGROUND: Allergic disorders are characterized by IgE antibody responses to a multitude of **allergens** as a result of the ability of these antibodies to specifically bind to high-affinity IgE receptors on mast cells and basophils. This interaction results in receptor activation and release of soluble mediators such as histamine and leukotrienes, which cause allergic reactions in various target organs. Because the synthesis of IgE is tightly regulated by cytokines and CD40 ligand (L) interactions, CD4+ helper T cells are obvious targets, with the aim to modulate **allergen**-induced IgE responses. OBJECTIVES: Because of the central role of **allergen**-specific T-helper type 2 (TH2) cells in the pathway leading to IgE synthesis in vitro and in vivo, we have evaluated the possibility of inhibiting **allergen**-induced activation of these cells by using **allergen**-derived peptides that have been modified by single **amino acid substitutions**. **METHODS**: Three cloned human TH2-like CD4+ T-cell lines, specific for Der p 1, the major **allergen** in house dust, were used in this study. Upon activation with Der p 1 or specific Der p 1-derived wild-type peptides, these T-cell clones produce high levels of IL-4 and IL-5 and low levels of interferon-gamma and IL-2, respectively, and furthermore give help to **B cells** for the production of IgE in vitro. Modified synthetic peptides were generated by the introduction of single **amino acid substitutions** into two different T-cell activation-inducing **epitopes** on Der p 1. The effects of these modified peptides were studied in Der p 1-induced proliferation, cytokine production, and in vitro IgE production assays. RESULTS: Several substituted Der p 1-derived peptides failed to induce T-cell proliferation, in contrast to the native peptides. In addition, some of these peptides acted as antagonists by strongly inhibiting wild-type peptide-induced proliferation as well as the production of interferon-gamma, IL-2, IL-4, and IL-5, although the production of the latter two cytokines was less affected than that of interferon-gamma, even

at a 100-fold molar excess of the antagonistic peptides. In addition, the presence of an excess of each of the antagonistic peptides during the activation of Der p I-specific T-cell clones prevented induction of CD40L expression, resulting in a failure of these cells to give help to **B cells** for the production of IgE in vitro, even in the presence of exogenous IL-4. CONCLUSIONS: Substitution of certain amino acid residues in immunogenic Der p 1-derived peptides results in the generation of peptides that fail to induce proliferation of Der p 1-specific T-cell clones. In addition, these modified peptides have strong antagonistic activities on Der p 1-induced proliferation, cytokine production, and CD40L expression by **allergen**-specific T-cell clones as well as on T cell-mediated IgE production by **B cells**. These findings suggest that modified peptides interfere with **allergen**-induced activation of T cells, including the production of cytokines and the expression of surface molecules important for successful T cell-**B cell** interactions, and may therefore have therapeutic potential by inhibiting the expansion and function of **allergen**-specific TH2 cells.

L10 ANSWER 4 OF 5 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.

1998:257635 Document No.: PREV199800257635. Antagonistic peptides specifically inhibit proliferation, cytokine production, CD40L expression, and help for IgE synthesis by Der p 1-specific human T-cell clones. Fasler, Stephan; Aversa, Gregoria; De Vries, Jan E.; Yssel, Hans (1). (1) INSERM U454, Hopital Arnaud de Villeneuve, 371 Ave. Doyen Gaston Giraud, 34295 Montpellier Cedex France. Journal of Allergy and Clinical Immunology, (April, 1998) Vol. 10, No. 4 PART 1, pp. 521-530. ISSN: 0091-6749. Language: English.

AB Background: Allergic disorders are characterized by IgE antibody responses to a multitude of **allergens** as a result of the ability of these antibodies to specifically bind to high-affinity IgE receptors on mast cells and basophils. This interaction results in receptor activation and release of soluble mediators such as histamine and leukotrienes, which cause allergic reactions in various target organs. Because the synthesis of IgE is tightly regulated by cytokines and CD40 ligand (L) interactions, CD4+ helper T cells are obvious targets, with the aim to modulate **allergen**-induced IgE responses. Objectives: Because of the central role of **allergen**-specific T-helper type 2 (TH2) cells in the pathway leading to IgE synthesis in vitro and in vivo, we have evaluated the possibility of inhibiting **allergen**-induced activation of these cells by using **allergen**-derived peptides that have been modified by single **amino acid substitutions**.
Methods: Three cloned human TH2-like CD4+ T-cell lines, specific for Der p 1, the major **allergen** in house dust, were used in this study. Upon activation with Der p 1 or specific Der p 1-dervived wild-type peptides, these T-cell clones produce high levels of IL-4 and IL-5 and low levels of interferon-gamma and IL-2, respectively, and furthermore give help to **B cells** for the production of IgE in vitro.
Modified synthetic peptides were generated by the introduction of single **amino acid substitutions** into two different T-cell activation-inducing **epitopes** on Der p 1. The effects of these modified peptides were studied in Der p 1-induced proliferation, cytokine production, and in vitro IgE production assays. Results: Several substituted Der p 1-derived peptides failed to induce T-cell proliferation, in contrast to the native peptides. In addition, some of these peptides acted as antagonists by strongly inhibiting wild-type peptide-induced proliferation as well as the production of interferon-gamma, IL-2, IL-4, and IL-5, although the production of the latter two cytokines was less affected than that of interferon-gamma, even at a 100-fold molar excess of the antagonistic peptides. In addition, the presence of an excess of each of the antagonistic peptides during the activation of Der p I-specific T-cell clones prevented induction of CD40L expression, resulting in a failure of these cells to give help to

B cells for the production of IgE in vitro, even in the presence of exogenous IL-4. Conclusions: Substitution of certain amino acid residues in immunogenic Der p 1-derived peptides results in the generation of peptides that fail to induce proliferation of Der p 1-specific T-cell clones. In addition, these modified peptides have strong antagonistic activities on Der p 1-induced proliferation, cytokine production, and CD40L expression by **allergen**-specific T-cell clones as well as on T cell-mediated IgE production by **B cells**. These findings suggest that modified peptides interfere with **allergen**-induced activation of T cells, including the production of cytokines and the expression of surface molecules important for successful T cell-**B cell** interactions, and may therefore have therapeutic potential by inhibiting the expansion and function of **allergen**-specific TH2 cells.

L10 ANSWER 5 OF 5 MEDLINE DUPLICATE 2

97322946 Document Number: 97322946. PubMed ID: 9179436. Localization of antigenic sites on Der p 2 using oligonucleotide-directed mutagenesis targeted to predicted surface residues. Smith A M; Chapman M D. (Department of Medicine, University of Virginia, Charlottesville 22908, USA.) CLINICAL AND EXPERIMENTAL ALLERGY, (1997 May) 27 (5) 593-9. Journal code: 8906443. ISSN: 0954-7894. Pub. country: ENGLAND: United Kingdom. Language: English.

AB BACKGROUND: Understanding the molecular nature of **allergen**-antibody interactions is important to understanding the mechanism of conventional immunotherapy as well as to designing alternative immunotherapeutic strategies. Many important **allergens** have been cloned and expressed, making it possible to apply recombinant DNA techniques to dissect antigenic determinants. OBJECTIVE: The aim of this study was to use predictive algorithms and site-directed mutagenesis to investigate monoclonal antibody and IgE antibody **epitopes** of the major house dust mite **allergen** Der p 2. **METHODS:** Computer algorithms were used to assess the primary amino acid sequence of Der p 2 and to identify regions of hydrophilic and flexible sequence. Subsequently, site-directed mutagenesis was used to generate **amino acid substitutions** at hydrophilic residues at positions 44-46 and at position 100. The variants were tested in a competitive inhibition ELISA with four group 2-specific murine monoclonal antibodies and with human IgE antibody from mite allergic patients. RESULTS: Conservative **amino acid substitutions** at position 44-46 did not distinguish IgE antibody **epitopes**, but did suggest that these residues are involved in the **epitope** defined by one monoclonal antibody, 15E11. Non-conservative substitution of proline at this position reduced binding to all four monoclonal antibodies, as well as IgE antibody, by 50-80%. Point mutants at position 100 mapped the **epitopes** of two monoclonal antibodies, 7A1 and 13A4, previously shown to bind the same region of Der p 2. In addition, the two variants tested at this position showed distinct inhibition curves with these two monoclonal antibodies indicating differences in fine specificity. CONCLUSIONS: Using predictive algorithms, in the absence of tertiary structural information, we have been able to localize important **B cell** determinants on Der p 2. The results suggest that it is possible to modulate antibody recognition of **allergens** using site-directed mutagenesis and that this approach may provide a new strategy for **allergen** specific immunotherapy.

=> s larsen h?/au
L13 1559 LARSEN H?/AU

=> s (ipsen h?/au or spangfort m?/au or lasrsen j?/au)
L14 437 (IPSEN H?/AU OR SPANGFORT M?/AU OR LASRSEN J?/AU)

=> s l14 and allergen
L15 267 L14 AND ALLERGEN

=> s l15 and B cell epitope
3 FILES SEARCHED...
L16 23 L15 AND B CELL EPITOPE

=> s l16 and determination
L17 0 L16 AND DETERMINATION

=> dup remove l16
PROCESSING COMPLETED FOR L16
L18 12 DUP REMOVE L16 (11 DUPLICATES REMOVED)

=> d l18 1-12 cbib abs

L18 ANSWER 1 OF 12 CAPLUS COPYRIGHT 2002 ACS
2002:391881 Document No. 136:400602 Mutant **allergens** with reduced IgE binding affinity and improved safety for specific allergy vaccination. Holm, Jens; **Ipsen, Henrik**; Nedergaard Larsen, Jorgen; **Spangfort, Michael Dho** (Alk-Abello A/S, Den.). PCT Int. Appl. WO 2002040676 A2 20020523, 210 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, CZ, DE, DE, DK, DK, DM, DZ, EC, EE, EE, ES, FI, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2001-DK764 20011116. PRIORITY: DK 2000-1718 20001116; US 2000-PV249361 20001116; US 2001-PV298170 20010614.

AB Novel recombinant **allergens** with multiple mutations and reduced IgE binding affinity are disclosed. The **allergens** are non-naturally occurring mutants of naturally-occurring **allergens**, including birch pollen Bet v 1 of Betula verrucosa, wasp venom Ves v 5 from Vespula vulgaris, house dust mite Der p 1 and Der p 2 from Dermatophagoides farinae and D. pteronyssinus, resp., and grass Phl p 5 from Phleum pratense. Site-directed mutagenesis and DNA shuffling is used to replace surface exposed residues of the **allergens** while the overall .alpha.-carbon backbone tertiary structure is essentially preserved. The inventive idea of the present idea is based on the recognition that a mutated **allergen** having IgE binding reducing mutations in multiple **B cell epitopes**, and at least one intact epitope, would on the one hand reduce the **allergen**-mediated crosslinking and on the other hand allow the raising of an IgG response with a binding ability competitive with that of IgE. The mutant **allergen** constitutes a highly advantageous **allergen** in that the risk of anaphylactic reactions is strongly reduced. X-ray crystallog. anal. of the three-dimensional structure is used to identify surface-exposed amino acid residues, and the retention of .alpha.-carbon backbone tertiary structure. Vaccination efficiency is measured by IgE binding, T cell proliferation assay, histamine release assays with human basophil, T cell reactivity based on proliferation and cytokine prodn., and induction of IgG antibodies and blocking antibodies.

L18 ANSWER 2 OF 12 MEDLINE DUPLICATE 1
2001448606 Document Number: 21240667. PubMed ID: 11342623. Recombinant **allergens** with reduced allergenicity but retaining immunogenicity of the natural **allergens**: hybrids of yellow jacket and paper wasp venom **allergen** antigen 5s. King T P; Jim S Y; Monsalve R I; Kagey-Sobotka A; Lichtenstein L M; **Spangfort M D**. (Rockefeller University, New York, NY 10021, USA.. kingtp@mail.rockefeller.edu) .

JOURNAL OF IMMUNOLOGY, (2001 May 15) 166 (10) 6057-65. Journal code: 2985117R. ISSN: 0022-1767. Pub. country: United States. Language: English.

AB The homologous venom **allergen** Ag 5s from the yellow jacket (Vespula vulgaris) and paper wasp (Polistes annularis) have 59% sequence identity of their respective 204 and 205 amino acid residues, and they have low degrees of antigenic cross-reactivity in insect allergic patients and in animal models. Hybrids containing different segments of these two vespid Ag 5s were expressed in yeast. Circular dichroism spectroscopy suggests the hybrids to have the secondary structure of natural Ag 5. Inhibition ELISA with human and murine Abs suggests the hybrids to have the discontinuous **B cell epitopes** of the natural Ag 5 but with an altered epitope density. The hybrids were immunogenic in mice for B and T cell responses to both Ag 5s. The N-terminal region of Ag 5 was found to contain its dominant **B cell epitope**(s). Hybrids containing 10-49 residues of yellow jacket Ag 5 showed 100- to 3000-fold reduction in allergenicity when tested by histamine release assay with basophils of yellow jacket-sensitive patients. Our findings suggest that hybrids represent a useful approach to map the discontinuous **B cell epitope**-containing regions of proteins. They also suggest that Ag 5 hybrids may be useful immunotherapeutic reagents in man.

L18 ANSWER 3 OF 12 CAPLUS COPYRIGHT 2002 ACS

2001:320966 Document No. 135:225746 Allerginicity of hybrid Ag 5s of yellow jacket and paper wasp venoms. King, T. P.; Kagey-Sobotka, A.; Jim, S.; Monsalve, R. I.; Lichtenstein, L. M.; **Spangfort, M. D.** (School of Medicine and ALK-Abello, Rockefeller University, Johns Hopkins University, New York, NY, 10021-6399, USA). International Archives of Allergy and Immunology, 124(1-3), 85-86 (English) 2001. CODEN: IAAIEG. ISSN: 1018-2438. Publisher: S. Karger AG.

AB Antigen (Ag) 5 is a major **allergen** of vaspid venoms. Homologous Ag 5s from yellow jacket (Vespula vulgaris) and paper wasp (Polistes annularis) have 59% sequence identity of their resp. 204- and 205-amino acid residues. These two Ag 5s, designated as Ves v 5 and Pol a 5, resp., have low degrees of antigenic cross-reactivity in insect-allergic patients and in animal models. The structure of Ves v 5 has been recently solved by x-ray crystallog. A study was conducted in which hybrids contg. different segments of these two vespid Ag 5s were prepd. by expression in yeast to study their immunol. properties. Findings illustrate that hybrid **allergens** can have a 100- to a 1000-fold redn. in allerginicity, yet retaining the immunogenicity of the natural **allergens**. This redn. in allerginicity of hybrids is due to a decrease of **B cell epitope** d. Each of the hybrids studied has only a portion of the B and T cell epitopes of Ves v 5, and a mixt. of the hybrids can, in principle, reconstitute the complete epitope library. Thus, this may be a useful approach to prep. modified **allergens** for use as vaccines as many **allergens** have sequence homol. wth proteins from other sources.

L18 ANSWER 4 OF 12 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE 2

2001:187735 Document No.: PREV200100187735. Dominant **B cell epitope**-containing region of a vespid **allergen** Ag 5t. King, P. (1); Jim, S. (1); Monsalve, R. I.; Kagey-Sobotka, A.; Lichtenstein, L. M.; **Spangfort, M. D.**. (1) Rockefeller University, New York, NY USA. Journal of Allergy and Clinical Immunology, (February, 2001) Vol. 107, No. 2, pp. S56. print. Meeting Info.: 57th Annual Meeting of the American Academy of Allergy, Asthma and Immunology New Orleans, Louisiana, USA March 16-21, 2001 ISSN: 0091-6749. Language: English. Summary Language: English.

L18 ANSWER 5 OF 12 MEDLINE DUPLICATE 3

2000318733 Document Number: 20318733. PubMed ID: 10861069. Dominant

epitopes and allergic cross-reactivity: complex formation between a Fab fragment of a monoclonal murine IgG antibody and the major **allergen** from birch pollen Bet v 1. Mirza O; Henriksen A; **Ipsen H;** Larsen J N; Wissenbach M; **Spangfort M D;** Gajhede M. (Protein Structure Group, Department of Chemistry, University of Copenhagen, Denmark.) JOURNAL OF IMMUNOLOGY, (2000 Jul 1) 165 (1) 331-8. Journal code: 2985117R. ISSN: 0022-1767. Pub. country: United States. Language: English.

AB The symptoms characteristic of allergic hypersensitivity are caused by the release of mediators, i.e., histamine, from effector cells such as basophils and mast cells. **Allergens** with more than one **B cell epitope** cross-link IgE Abs bound to high affinity FcepsilonRI receptors on mast cell surfaces leading to aggregation and subsequent mediator release. Thus, **allergen**-Ab complexes play a crucial role in the cascade leading to the allergic response. We here report the structure of a 1:1 complex between the major birch pollen **allergen** Bet v 1 and the Fab fragment from a murine monoclonal IgG1 Ab, BV16, that has been solved to 2.9 A resolution by x-ray diffraction. The mAb is shown to inhibit the binding of allergic patients' IgE to Bet v 1, and the **allergen**-IgG complex may therefore serve as a model for the study of **allergen**-IgE interactions relevant in allergy. The size of the BV16 epitope is 931 A2 as defined by the Bet v 1 Ab interaction surface. Molecular interactions predicted to occur in the interface are likewise in agreement with earlier observations on Ag-Ab complexes. The epitope is formed by amino acids that are conserved among major **allergens** from related species within the Fagales order. In combination with a surprisingly high inhibitory capacity of BV16 with respect to allergic patients' serum IgE binding to Bet v 1, these observations provide experimental support for the proposal of dominant IgE epitopes located in the conserved surface areas. This model will facilitate the development of new and safer vaccines for **allergen** immunotherapy in the form of mutated **allergens**.

L18 ANSWER 6 OF 12 CAPLUS COPYRIGHT 2002 ACS

2001:32068 Document No. 135:59797 Structural and biological demands on recombinant **allergens** related to their application. **Spangfort, M. D.** (Germany). Arbeiten aus dem Paul-Ehrlich-Institut (Bundesamt fuer Sera und Impfstoffe) Langen, 93(Regulatory Control and Standardization of Allergenic Extracts), 197-202 (English) 2000. CODEN: APGFEK. ISSN: 0936-8671. Publisher: GIT Verlag GmbH.

AB A review with 9 refs. Topics discussed include the protein folding problem; conformational **B cell epitopes**; and the modulation of IgE-reactivity by site-directed mutagenesis.

L18 ANSWER 7 OF 12 CAPLUS COPYRIGHT 2002 ACS

1999:614141 Document No. 131:241995 Mutant recombinant **allergens** for use as allergy vaccines. **Ipsen, Hans Henrik;** **Spangfort, Michael Dho;** Larsen, Jorgen Nedergaard (Alk-Abello A/S, Den.). PCT Int. Appl. WO 9947680 A1 19990923, 77 pp. DESIGNATED STATES: W: AE, AL, AM, AT, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, CZ, DE, DE, DK, DK, EE, EE, ES, FI, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1999-DK136 19990316. PRIORITY: DK 1998-364 19980316.

AB Novel recombinant **allergens** are disclosed. The **allergens** are non-naturally occurring mutants derived from naturally-occurring **allergens**. The overall .alpha.-carbon backbone tertiary structure is essentially preserved. Also disclosed are methods for prepg. such recombinant **allergens** as well as uses

thereof. The invention is based on the idea that the mechanism of successful allergy vaccination is not an alteration of the ongoing Th2-type immune response, but rather a parallel initiation of a new Th1-type immune response involving tertiary epitope recognition by B-cells and antibody formation. Addnl., dominant IgE binding epitopes are proposed. These epitopes are supposed to be constituted by tertiary structure dependent coherent surface areas large enough to accommodate antibody binding and conserved among isoallergens, variants, and/or homologous **allergens** from related species. Mutant forms of Bet v 1 and Ves v 5 **allergens** were produced. The Bet v 1 mutants displayed reduced IgE binding although the tertiary structure of the wild-type Bet v 1 **allergen** was retained. A "triple-patch mutant" of Bet v 1 was able to induce proliferation in T cell lines from 3 different birch pollen allergic patients with stimulation indexes similar to recombinant and naturally occurring Bet v 1.

L18 ANSWER 8 OF 12 SCISEARCH COPYRIGHT 2002 ISI (R)
1998:176778 The Genuine Article (R) Number: YW339. Cross-reactive **B -cell epitopes** on the Betulaceae pollen major **allergens** identified by 3-D structural analysis and in vitro site directed mutagenesis of Bet v 1.. Larsen J N (Reprint); Schou C; **Ipsen H; Spangfort M D**. ABELLO, ALK, HORSHOLM, DENMARK. JOURNAL OF ALLERGY AND CLINICAL IMMUNOLOGY (JAN 1998) Vol. 101, No. 1, Part 2, pp. 951-951. Publisher: MOSBY-YEAR BOOK INC. 11830 WESTLINE INDUSTRIAL DR, ST LOUIS, MO 63146-3318. ISSN: 0091-6749. Pub. country: DENMARK. Language: English.

L18 ANSWER 9 OF 12 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
1998:154659 Document No.: PREV199800154659. Cross-reactive **B-cell epitopes** on the Betulaceae pollen major **allergens** identified by 3-D structural analysis and in vitro site directed mutagenesis of Bet v 1. Larsen, J. N.; Schou, C.; **Ipsen, H.; Spangfort, M. D.**. ALK-ABELLO, Horsholm Denmark. Journal of Allergy and Clinical Immunology, (Jan., 1998) Vol. 101, No. 1 PART 2, pp. S229. Meeting Info.: 54th Annual Meeting of the American Academy of Allergy, Asthma and Immunology Washington, DC, USA March 13-18, 1998 American Academy of Allergy, Asthma, and Immunology. ISSN: 0091-6749. Language: English.

L18 ANSWER 10 OF 12 MEDLINE DUPLICATE 4
97102431 Document Number: 97102431. PubMed ID: 8946858. X-ray and NMR structure of Bet v 1, the origin of birch pollen allergy. Gajhede M; Osmark P; Poulsen F M; **Ipsen H**; Larsen J N; Joost van Neerven R J; Schou C; Lowenstein H; **Spangfort M D**. (Department of Chemistry, University of Copenhagen, Denmark.) NATURE STRUCTURAL BIOLOGY, (1996 Dec) 3 (12) 1040-5. Journal code: 9421566. ISSN: 1072-8368. Pub. country: United States. Language: English.
AB The three-dimensional structure of the major birch pollen **allergen**, the 17,500 M(r) acidic protein Bet v 1 (from the birch, Betula verrucosa), is presented as determined both in the crystalline state by X-ray diffraction and in solution by nuclear magnetic resonance (NMR) spectroscopy. This is the first experimentally determined structure of a clinically important inhalant major **allergen**, estimated to cause allergy in 5-10 million individuals worldwide. The structure shows three regions on the molecular surface predicted to harbour cross-reactive **B-cell epitopes** which provide a structural basis for the allergic symptoms that birch pollen allergic patients show when they encounter pollens from related trees such as hazel, alder and hornbeam. The structure also shows an unusual feature, a 30 A-long forked cavity that penetrates the entire protein.

L18 ANSWER 11 OF 12 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
1997:26116 Document No.: PREV199799325319. Characterization of purified

recombinant Bet v 1 with authentic N-terminus, cloned in fusion with maltose-binding protein. **Spangfort, Michael D. (1); Ipsen, Henrik;** Sparholt, Susanne H.; Aasmul-Olsen, Stig; Larsen, Martin R.; Mortz, Ejvind; Roepstorff, Peter; Larsen, Jorgen N.. (1) Res. Dep., ALK-ABELLO Group, Boge Alle 10-12, DK-2970 Horsholm Denmark. Protein Expression and Purification, (1996) Vol. 8, No. 3, pp. 365-373. ISSN: 1046-5928. Language: English.

AB A gene encoding the pollen major **allergen** Bet v 1 from Betula verrucosa (White Birch) has been cloned and expressed in Escherichia coli as a fusion with maltose-binding protein and a Factor Xa proteolytic cleavage site. A generally applicable cloning strategy based on polymerase chain reaction was designed to position the Factor Xa proteolytic site so that the authentic amino terminus of Bet v 1 was generated after cleavage. Fusion protein was isolated by amylose affinity chromatography and enzymatically cleaved by incubation with Factor Xa. Recombinant Bet v 1 was isolated by gel filtration and gave rise to a single band with apparent molecular weight of 17 kDa when analyzed by SDS-polyacrylamide gel electrophoresis. N-terminal sequencing of the first 20 amino acids showed complete agreement with the deduced Bet v 1 DNA sequence. Mass spectrometry showed that recombinant Bet v 1 has a molecular mass of 17,440 +- 2 Da; 86% of the recombinant Bet v 1 amino acid sequence could be verified by digestion with Lys-C and mass spectrometric peptide mapping. The yield of purified recombinant Bet v 1 was 10 mg per liter E. coli cell culture. Two-dimensional gel electrophoresis of purified recombinant protein gave rise to one major protein spot and one or two minor spots focusing at slightly different pHs. The immunochemical properties of recombinant protein were indistinguishable from those of naturally occurring Bet v 1 when compared using a panel of murine monoclonal antibodies and serum IgE from birch pollen allergic patients. Furthermore, recombinant Bet v 1 elicited T-cell proliferation comparable to that of natural Bet v 1. Thus, the methods used for bacterial expression and protein purification result in relatively high yields of folded recombinant Bet v 1 with correct N-terminal sequence and molecular mass. Furthermore, the B- and T-cell epitope structures of recombinant Bet v 1 closely resemble those of the natural protein from pollen.

L18 ANSWER 12 OF 12 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
1994:150524 Document No.: PREV199497163524. Characterization of purified recombinant Bet v I, produced by fed-batch fermentation. Larsen, J. N.; Casals, A. B.; From, N. B.; **Ipsen, H.;** Klysner, S.. Horsholm Denmark. Journal of Allergy and Clinical Immunology, (1994) Vol. 93, No. 1 PART 2, pp. 230. Meeting Info.: Fiftieth Annual Meeting of the American Academy of Allergy and Immunology Anaheim, California, USA March 4-9, 1994 ISSN: 0091-6749. Language: English.

```
=> s b cell epitope
   3 FILES SEARCHED...
L19       5760 B CELL EPITOPE

=> s l19 and allergen
L20        482 L19 AND ALLERGEN

=> s l20 and 70% identity
L21          0 L20 AND 70% IDENTITY

=> s l20 and 400 angstrome
L22          0 L20 AND 400 ANGSTROME

=> dup remove l20
PROCESSING COMPLETED FOR L20
L23         183 DUP REMOVE L20 (299 DUPLICATES REMOVED)
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=> s l23 and IgE binding mutant
L24 0 L23 AND IGE BINDING MUTANT

=> s l23 and IgE
L25 112 L23 AND IGE

=> s l25 and binding
L26 77 L25 AND BINDING

=> s l26 and amino acid substitution
L27 2 L26 AND AMINO ACID SUBSTITUTION

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PROCESSING COMPLETED FOR L27
L28 2 DUP REMOVE L27 (0 DUPLICATES REMOVED)

=> d l28 1-2 cbib abs

L28 ANSWER 1 OF 2 SCISEARCH COPYRIGHT 2002 ISI (R)
2000:153581 The Genuine Article (R) Number: 285RQ. Mutational analysis of the **IgE-binding** epitopes of P34/Gly m Bd 30K. Helm R M (Reprint); Cockrell G; Connaughton C; West C M; Herman E; Sampson H A; Bannon G A; Burks A W. UNIV ARKANSAS MED SCI, ARKANSAS CHILDRENS NUTR CTR, DEPT PEDIAT, 1120 MARSHALL ST, LITTLE ROCK, AR 72202 (Reprint); UNIV ARKANSAS MED SCI, DEPT BIOCHEM & MOL BIOL, LITTLE ROCK, AR 72202; AGR RES SERV, CLIMATE STRESS LAB, USDA, BELTSVILLE, MD; MT SINAI SCH MED, DIV PEDIAT ALLERGY & IMMUNOL, NEW YORK, NY. JOURNAL OF ALLERGY AND CLINICAL IMMUNOLOGY (FEB 2000) Vol. 105, No. 2, Part 1, pp. 378-384. Publisher: MOSBY-YEAR BOOK INC. 11830 WESTLINE INDUSTRIAL DR, ST LOUIS, MO 63146-3318 . ISSN: 0091-6749. Pub. country: USA. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Background: Peanuts and soybeans are 2 foods that have been shown to be responsible for many atopic disorders. Because of their nutritional benefit, soybean proteins are now being used increasingly in a number of food products. Previous studies have documented multiple **allergens** in soybean extracts, including glycinin, beta-conglycinin, and the P34/Gly m Bd 30K protein.

Objective: Our overall goal was to identify soybean-specific **allergens** to begin to understand molecular and immunochemical characteristics of legume proteins. The specific aim of the current investigation was to identify the essential amino acid residues necessary for **IgE binding** in the 5 distinct immunodominant epitopes of P34/Gly m Ed 30K,

Methods: Serum **IgE** from 6 clinically sensitive soybean-allergic individuals was used to identify P34/Gly m Ed 30K in the native and single amino acid substituted peptides with use of the SPOTS peptide synthesis technique to determine critical amino acids required for **IgE binding**.

Results: The intensity of **IgE binding** and epitope recognition by serum **IgE** from the individuals varied substantially. With use of serum from 6 clinically soybean-sensitive individuals, 2 of the 5 immunodominant epitopes could be mutagenized to non-**IgE binding** peptides,

Conclusions: Single-site **amino acid substitution** of the 5 immunodominant epitopes of Gly m Ed 30K with alanine revealed that **IgE binding** could be reduced or eliminated in epitopes 6 and 16 in the serum obtained from 6 soybean-sensitive patients.

L28 ANSWER 2 OF 2 MEDLINE
2001086248 Document Number: 20564013. PubMed ID: 11112857. A soybean G2 glycinin **allergen**. 2. Epitope mapping and three-dimensional modeling. Helm R M; Cockrell G; Connaughton C; Sampson H A; Bannon G A;

Beilinson V; Nielsen N C; Burks A W. (Department of Pediatrics, University of Arkansas for Medical Sciences, Arkansas Children's Nutrition Center, Little Rock, AR 72202-3591, USA.) INTERNATIONAL ARCHIVES OF ALLERGY AND IMMUNOLOGY, (2000 Nov) 123 (3) 213-9. Journal code: 9211652. ISSN: 1018-2438. Pub. country: Switzerland. Language: English.

AB BACKGROUND: Multiple **allergens** have been documented in soybean extracts. **IgE** from individuals allergic to soybeans, but not to peanut, has been shown by immunoblot analysis to bind to proteins with a molecular weight of approximately 22 kD. These findings suggested that this unique protein fraction from soybean might be responsible, in part, for soybean allergic reactivity. The objective of the present study was to characterize specific **B cell epitopes**, to determine if any amino acid was critical to **IgE binding** and to model the 22-kD G2 soybean **allergen** to the three-dimensional (3-D) phaseolin molecule. METHODS: **B cell epitopes** were identified using SPOTs peptide analysis. Structural orientation of the **IgE-binding** regions was mapped to the 3-D phaseolin molecule using molecular modeling of the protein tertiary structure. RESULTS: Eleven linear epitopes, representing 15 amino acid peptide sequences, bound to **IgE** in the glycinin molecule. These epitopes were predicted to be distributed asymmetrically on the surface of G2 trimers. CONCLUSIONS: Only 1 epitope could be rendered non-**IgE binding** by alanine substitutions in the peptide. The nonrandom distribution of the **IgE binding** sites provides new insight into their organization in trimers in 11S complexes of the G2 glycinin **allergen**.


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PROCESSING COMPLETED FOR L27
L29             2 DUP REMOVE L27 (0 DUPLICATES REMOVED)

=> d his

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     14 AUG 2002
L1              0 S B CELL EPITOPE DETERMINATION
L2       10218462 S METHOD
L3          25207 S L2 AND ALLERGEN
L4            583 S L3 AND B CELL
L5              0 S L4 AND EPITOPE DETERMINATION
L6            256 S L4 AND EPITOPE
L7              0 S L6 AND ANGSTROM
L8              0 S L6 AND SURFACE EXPOSURE
L9             10 S L6 AND AMINO ACID SUBSTITUTION
L10             5 DUP REMOVE L9 (5 DUPLICATES REMOVED)
L11             2 S L10 AND IGE BINDING
L12             2 DUP REMOVE L11 (0 DUPLICATES REMOVED)
L13          1559 S LARSEN H?/AU
L14           437 S (IPSEN H?/AU OR SPANGFORT M?/AU OR LASRSEN J?/AU)
L15           267 S L14 AND ALLERGEN
L16            23 S L15 AND B CELL EPITOPE
L17             0 S L16 AND DETERMINATION
L18            12 DUP REMOVE L16 (11 DUPLICATES REMOVED)
L19          5760 S B CELL EPITOPE
L20           482 S L19 AND ALLERGEN
L21             0 S L20 AND 70% IDENTITY
L22             0 S L20 AND 400 ANGSTROME
L23           183 DUP REMOVE L20 (299 DUPLICATES REMOVED)
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L24           0 S L23 AND IGE BINDING MUTANT
L25         112 S L23 AND IGE
L26          77 S L25 AND BINDING
L27           2 S L26 AND AMINO ACID SUBSTITUTION
L28           2 DUP REMOVE L27 (0 DUPLICATES REMOVED)
L29           2 DUP REMOVE L27 (0 DUPLICATES REMOVED)

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PROCESSING COMPLETED FOR L26
L30          77 DUP REMOVE L26 (0 DUPLICATES REMOVED)

=> d l30 1-77 cbib abs

L30  ANSWER 1 OF 77  BIOSIS  COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
2002:278076  Document No.: PREV200200278076. Tolerogenic fragments of natural
     allergens. Berrens, Lubertus (1); Gonzales Romano, Maria Leticia;
     Gallego Camara, Maria Teresa. (1) Utrecht Netherlands. ASSIGNEE: C.B.F.
     Leti, S.A., Tres Cantos, Spain. Patent Info.: US 6350590 February 26,
     2002. Official Gazette of the United States Patent and Trademark Office
     Patents, (Feb. 26, 2002) Vol. 1255, No. 4, pp. No Pagination.
     http://www.uspto.gov/web/menu/patdata.html. e-file. ISSN: 0098-1133.
     Language: English.
AB   The present invention relates to a process for the controlled enzymatic
     cleavage of purified and depigmented active allergenic proteins from
     indoor and outdoor source materials, which process produces fragments of
     allergens that retain the the natural T-lymphocyte stimulating
     epitopes, but are depleted of IgE-binding B-
     cell epitopes and complement-activating agents. The
     invention also relates to the new pharmaceutical products. These
     allergen fragments do not exhibit the disadvantages of
     conventional allergenic extracts for immunotherapy and can be safely used
     to induce a state of specific T-cell anergy and immunological tolerance in
     allergic human beings.

L30  ANSWER 2 OF 77  CAPLUS  COPYRIGHT 2002 ACS
2002:391881  Document No. 136:400602  Mutant allergens with reduced
     IgE binding affinity and improved safety for specific
     allergy vaccination.  Holm, Jens; Ipsen, Henrik; Nedergaard Larsen,
     Jorgen; Spangfort, Michael Dho (Alk-Abello A/S, Den.).  PCT Int. Appl. WO
     2002040676 A2 20020523, 210 pp.  DESIGNATED STATES: W:  AE, AG, AL, AM,
     AT, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, CZ,
     DE, DE, DK, DK, DM, DZ, EC, EE, EE, ES, FI, FI, GB, GD, GE, GH, GM, HR,
     HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV,
     MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE,
     SG, SI, SK, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM,
     ZW, AM, AZ, BY, KG, KZ; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE,
     DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN,
     TD, TG, TR.  (English).  CODEN: PIXXD2.  APPLICATION: WO 2001-DK764
     20011116.  PRIORITY: DK 2000-1718 20001116; US 2000-PV249361 20001116; US
     2001-PV298170 20010614.
AB   Novel recombinant allergens with multiple mutations and reduced
     IgE binding affinity are disclosed.  The
     allergens are non-naturally occurring mutants of
     naturally-occurring allergens, including birch pollen Bet v 1 of
     Betula verrucosa, wasp venom Ves v 5 from Vespula vulgaris, house dust
     mite Der p 1 and Der p 2 from Dermatophagoides farinae and D.
     pteronyssinus, resp., and grass Phl p 5 from Phleum pratense.
     Site-directed mutagenesis and DNA shuffling is used to replace surface
     exposed residues of the allergens while the overall
     .alpha.-carbon backbone tertiary structure is essentially preserved.  The
     inventive idea of the present idea is based on the recognition that a
     mutated allergen having IgE binding reducing
     mutations in multiple B cell epitopes, and
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at least one intact epitope, would on the one hand reduce the **allergen**-mediated crosslinking and on the other hand allow the raising of an IgG response with a **binding** ability competitive with that of **IgE**. The mutant **allergen** constitutes a highly advantageous **allergen** in that the risk of anaphylactic reactions is strongly reduced. X-ray crystallog. anal. of the three-dimensional structure is used to identify surface-exposed amino acid residues, and the retention of .alpha.-carbon backbone tertiary structure. Vaccination efficiency is measured by **IgE binding**, T cell proliferation assay, histamine release assays with human basophil, T cell reactivity based on proliferation and cytokine prodn., and induction of IgG antibodies and blocking antibodies.

L30 ANSWER 3 OF 77 SCISEARCH COPYRIGHT 2002 ISI (R)
2002:510241 The Genuine Article (R) Number: 561MT. Analysis of **IgE** antibodies from a patient with atopic dermatitis: Biased V gene usage and evidence for polyreactive **IgE** heavy chain complementarity-determining region 3. Edwards M R; Brouwer W; Choi C H Y; Ruhno J; Ward R L; Collins A M (Reprint). Univ New S Wales, Sch Microbiol & Immunol, Sydney, NSW 2052, Australia (Reprint); Univ New S Wales, Sch Microbiol & Immunol, Kensington, NSW 2033, Australia; Univ New S Wales, Sch Med, Kensington, NSW 2033, Australia; Royal N Shore Hosp, St Leonards, NSW 2065, Australia; St Vincents Hosp, Dept Med Oncol, Darlinghurst, NSW 2010, Australia. JOURNAL OF IMMUNOLOGY (15 JUN 2002) Vol. 168, No. 12, pp. 6305-6313. Publisher: AMER ASSOC IMMUNOLOGISTS. 9650 ROCKVILLE PIKE, BETHESDA, MD 20814 USA. ISSN: 0022-1767. Pub. country: Australia. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS
AB To better understand V gene usage, specificity, and clonal origins of **IgE** Abs in allergic reactions, we have constructed a combinatorial Ab library from the mRNA of an adult patient with atopic dermatitis. Sequence analysis of random clones revealed that 33% of clones used the IGHV6-1 H chain V gene segment, the only member of the V(H)6 gene family. IGHV6-1 is rarely used in the expressed adult repertoire; however, it is associated with fetal derived Abs. Features of the V(H)6 rearrangements included short complementarity-determining region 3, frequent use of IGHD7-27 D gene, and little nucleotide addition at the D-J junction. There was also a low level of mutation compared with V(H)1, V(H)3, and V(H)4 rearrangements. The library was expressed as phage-Fab fusions, and specific phage selected by panning on the egg **allergen** ovomucoid. Upon expression as soluble **IgE** Fabs, 12 clones demonstrated **binding** to ovomucoid, skim milk, and BSA by ELISA. Nucleotide sequencing demonstrated that the IGHV6-1 V gene segment encoded each of the 12 multiply reactive **IgE** Fabs. A cyclic peptide was designed from the complementarity-determining region 3 of several of these clones. The cyclic peptide bound both self and nonself Ags, including ovomucoid, human IgG, tetanus toxoid, and human and bovine von Willebrand factor. These results suggest that some **IgE** Abs may bind more than one Ag, which would have important implications for understanding the multiple sensitivities seen in conditions such as atopic dermatitis.

L30 ANSWER 4 OF 77 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.
2002175159 EMBASE [Immunotherapy: Is there a place for recombinant **allergens**?]. DESENSIBILISATION: Y A-T-IL UNE PLACE POUR LES ALLERGENES RECOMBINANTS?. Pauli G.. G. Pauli, Faculte de Medecine, Universite Louis-Pasteur, Inserum U425 et Serv. de Pneumol., BP 426, 67091 Strasbourg Cedex, France. Gabrielle.Pauli@chru-strasbourg.fr. Revue Francaise d'Allergologie et d'Immunologie Clinique 42/3 (335-342) 2002. Refs: 52.
ISSN: 0335-7457. CODEN: RFAIBB. Pub. Country: France. Language: French. Summary Language: English; French.
AB Specific immunotherapy is the sole etiologic mode of treatment in allergic diseases, leading to modulation of the immune response against

allergens. Extracts of natural **allergen** sources currently used in clinical practice are heterogeneous and their composition and content in major and minor **allergens** are variable. Cloning of allergenic proteins is the starting point for the development of recombinant **allergens** offering new therapeutic perspectives in immunotherapy. In conventional immunotherapy the use of recombinant **allergens** should allow the adequate adaptation of immunotherapy to the specific **IgE** sensitization of each allergic patient and the quantification of the dosage of major and minor **allergens** in **allergen** preparations. The use of peptides and engineered hypoallergens for immunotherapy should lead to better efficacy and greater safety by reducing the risk of anaphylactic side reactions. Over the last 5 years, several studies have been performed with peptides and modified recombinant **allergens** derived from the most important **allergens**, using various approaches: treatment with peptides representing a portion of T cell epitopes, selection of isoforms of **allergen** molecules identified on the basis of low/no **IgE binding** activity, production of hypoallergenic derivatives using recombinant technology (allergenic fragments and recombinant oligomers where conformational B epitopes are lost or hidden, modification of important amino-acid sequences by site-directed mutagenesis and destruction of disulfide bonds either in **IgE binding** epitopes or at sites outside the epitopes influencing the conformation of the molecules and thereby the **IgE binding** activity), synthesis of peptides corresponding to portions of **B cell epitopes**. All these engineered hypoallergens have been selected by experimental animal studies and in vitro studies using T lymphocytes from allergic patients, and sera to assess loss of **IgE** reactivities by RAST-EIA and **IgE** inhibition assay; in vivo studies were also performed in sensitized patients using cutaneous tests. Desensitization efficacy studies were only performed with peptides derived from T cell epitopes. .COPYRGT. 2002 Editions scientifiques et medicales Elsevier SAS.

L30 ANSWER 5 OF 77 SCISEARCH COPYRIGHT 2002 ISI (R)

2002:523298 The Genuine Article (R) Number: 565KX. **IgE binding** conformational epitopes of Asp f 3, a major **allergen** of Aspergillus fumigatus. Ramachandran H; Jayaraman V; Banerjee B; Greenberger P A; Kelly K J; Fink J N; Kurup V P (Reprint). Vet Affairs Med Ctr, Res Serv 151I, 5000 W Natl Ave, Milwaukee, WI 53295 USA (Reprint); Vet Affairs Med Ctr, Res Serv, Milwaukee, WI 53295 USA; Med Coll Wisconsin, Dept Pediat, Div Allergy Immunol, Milwaukee, WI 53226 USA; Marquette Univ, Dept Chem, Milwaukee, WI 53233 USA; Northwestern Univ, Sch Med, Div Allergy Immunol, Chicago, IL USA. CLINICAL IMMUNOLOGY (JUN 2002) Vol. 103, No. 3, Part 1, pp. 324-333. Publisher: ACADEMIC PRESS INC ELSEVIER SCIENCE. 525 B ST, STE 1900, SAN DIEGO, CA 92101-4495 USA. ISSN: 1521-6616. Pub. country: USA. Language: English.

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB sp, f 3 has been identified as one of the major **allergens** of Aspergillus fumigatus associated with the sensitization and immune responses in allergic bronchopulmonary aspergillosis (ABPA). In order to understand the structure/function relationship of Asp f 3, we studied synthetic peptides and constructed mutants deleted of specific **IgE binding** regions. The mutated **allergens** were obtained by expressing the genes and studied by ELISA for their reactivity with **IgE** from patients with ABPA. Seven linear **IgE binding** regions spanning the whole Asp f 3 molecule were demonstrated. The results demonstrated strong **binding** of **IgE** from ABPA patients with Asp f 3 and one mutant, Asp f 3(1-150), but not with other mutant constructs. The results identified 12 amino acids at the N-terminal end and 8 amino acids (143-150) at the C-terminal end as significant in the conformational constraints for **IgE binding**. The Fourier transfer spectra showed

comparable P-sheet structure of Asp f 31-150 and Asp f 3, indicating the role of secondary structure in **IgE binding**. The primary and secondary structures may help understanding of the functional role the **allergens** play in the disease and may have implications in immunodiagnosis and probably immunotherapy. (C) 2002 Elsevier Science (USA).

L30 ANSWER 6 OF 77 MEDLINE

2002373866 Document Number: 22115009. PubMed ID: 12119498. Genetic engineering of **allergens**: future therapeutic products. Ferreira F; Wallner M; Breiteneder H; Hartl A; Thalhamer J; Ebner C. (Institute of Genetics, University of Salzburg, Salzburg, Austria.) INTERNATIONAL ARCHIVES OF ALLERGY AND IMMUNOLOGY, (2002 Jul) 128 (3) 171-8. Journal code: 9211652. ISSN: 1018-2438. Pub. country: Switzerland. Language: English.

AB Genetic engineering of **allergens** for specific immunotherapy should aim at the production of modified molecules with reduced **IgE-binding** epitopes (hypoallergens), while preserving structural motifs necessary for T cell recognition (T cell epitopes) and for induction of IgG antibodies reactive with the natural **allergen** (blocking antibodies). Common approaches for engineering of hypoallergens usually require knowledge of T and **B cell epitopes** and involve changing specific base pairs (mutated gene), introduction of a new piece of DNA into the existing DNA molecule (chimeric or hybrid gene), and deletions (truncated gene or fragments). DNA family shuffling has the advantage that it does not require a priori knowledge of structural and functional properties for efficient generation of hypoallergens. The combination of the hypoallergen concept with the Th1-inducing genetic immunization approach might be an attractive alternative for protein-based immunotherapy.

L30 ANSWER 7 OF 77 MEDLINE

2001490137 Document Number: 21423541. PubMed ID: 11511525. Nonanaphylactic synthetic peptides derived from **B cell epitopes** of the major grass pollen **allergen**, Phl p 1, for allergy vaccination. Focke M; Mahler V; Ball T; Sperr W R; Majlesi Y; Valent P; Kraft D; Valenta R. (Department of Pathophysiology, Vienna General Hospital, AKH, University of Vienna, Waehringer Guertel 18-20, A-1090 Vienna, Austria.) FASEB JOURNAL, (2001 Sep) 15 (11) 2042-4. Journal code: 8804484. ISSN: 0892-6638. Pub. country: United States. Language: English.

AB Worldwide more than 200 million individuals are allergic to group 1 grass pollen **allergens**. We have used the major timothy grass pollen **allergen** Phl p 1, which cross-reacts with most grass-, corn-, and monocot-derived group 1 **allergens** to develop a generally applicable strategy for the production of hypoallergenic allergy vaccines. On the basis of the experimentally determined **B cell epitopes** of Phl p 1, we have synthesized five synthetic peptides. These peptides are derived from the major Phl p 1 **IgE** epitopes and were between 28-32 amino acids long. We demonstrate by nuclear magnetic resonance that the peptides exhibit no secondary and tertiary structure and accordingly failed to bind **IgE** antibodies from grass pollen allergic patients. The five peptides, as well as an equimolar mixture thereof, lacked allergenic activity as demonstrated by basophil histamine release and skin test experiments in grass pollen allergic patients. When used as immunogens in mice and rabbits, the peptides induced protective IgG antibodies, which recognized the complete Phl p 1 wild-type **allergen** and group 1 **allergens** from other grass species. Moreover, peptide-induced antibodies inhibited the **binding** of grass pollen allergic patients **IgE** antibodies to the wild-type **allergen**. We thus demonstrate that synthetic hypoallergenic peptides derived from **B cell**

epitopes of major **allergens** represent safe vaccine candidates for the treatment of **IgE**- mediated allergies.

L30 ANSWER 8 OF 77 MEDLINE
2001459657 Document Number: 21149980. PubMed ID: 11251633. Immunological and molecular characterization of the major **allergens** from lilac and privet pollens overproduced in Pichia pastoris. Gonzalez E; Villalba M; Rodriguez R. (Departamento de Bioquimica y Biologia Molecular, Facultad de Quimica, Universidad Complutense, 28040 Madrid, Spain.) CLINICAL AND EXPERIMENTAL ALLERGY, (2001 Feb) 31 (2) 313-21. Journal code: 8906443. ISSN: 0954-7894. Pub. country: England: United Kingdom. Language: English.
AB The main **allergens** from privet and lilac pollens, Lig v 1 and Syr v 1, are proteins homologous to Ole e 1 and have been shown to be involved in cross-reactivity. To overproduce the correctly folded Lig v 1 and Syr v 1 **allergens** and to study their immunological properties in comparison with those of their natural counterparts. The yeast Pichia pastoris was used as an expression system to produce these recombinant **allergens**. The proteins were isolated by ion-exchange and size-exclusion chromatographies. Amino acid quantifying, Edman degradation, mass spectrometry and circular dichroism were carried out to obtain molecular properties of the recombinant proteins. Anti-Ole e 1 monoclonal and polyclonal antibodies, as well as sera from patients allergic to olive pollen, were used in immunoblotting and ELISA for immunological characterization. Recombinant Lig v 1 and Syr v 1 were secreted at high yield to the extracellular medium of the yeast. The purified proteins displayed the native conformation, as deduced from their spectroscopic properties and **binding** ability to an IgG monoclonal antibody. The recombinant **allergens** behaved similarly to their natural counterparts when they were analysed against Ole e 1-specific antibodies. **IgE** and IgG **binding** properties of lilac and privet **allergens** to olive allergic sera and Ole e 1-specific antibodies indicated that these molecules share common **B-cell epitopes** with Ole e 1. P. pastoris yeast is an appropriate system for the efficient production of Ole e 1-like **allergens**, which could be used as analogous **allergens** and predictors of clinical sensitization.

L30 ANSWER 9 OF 77 SCISEARCH COPYRIGHT 2002 ISI (R)
2001:180456 The Genuine Article (R) Number: 403YV. Significance of **IgE-binding** epitopes in allergic disease. Bufe A (Reprint). Ruhr Univ Bochum, Bergmannsheil, Burkle-de-la-Camp Pl 1, D-44789 Bochum, Germany (Reprint); Ruhr Univ Bochum, Bergmannsheil, D-44789 Bochum, Germany. JOURNAL OF ALLERGY AND CLINICAL IMMUNOLOGY (FEB 2001) Vol. 107, No. 2, pp. 219-221. Publisher: MOSBY, INC. 11830 WESTLINE INDUSTRIAL DR, ST LOUIS, MO 63146-3318 USA. ISSN: 0091-6749. Pub. country: Germany. Language: English.

L30 ANSWER 10 OF 77 SCISEARCH COPYRIGHT 2002 ISI (R)
2001:645318 The Genuine Article (R) Number: 460PV. Nonanaphylactic synthetic peptides derived from **B cell epitopes** of the major grass pollen **allergen**, Phl p 1, for allergy vaccination. Focke M; Mahler V; Ball T; Sperr W R; Majlesi Y; Valent P; Kraft D; Valenta R (Reprint). Univ Vienna, Vienna Gen Hosp, AKH, Dept Pathophysiol, Mol Immunopathol Grp, Waehringer Guertel 18-20, A-1090 Vienna, Austria (Reprint); Univ Vienna, Vienna Gen Hosp, AKH, Dept Pathophysiol, Mol Immunopathol Grp, A-1090 Vienna, Austria; Univ Vienna, Vienna Gen Hosp, AKH, Dept Hematol & Hemostaseol, A-1090 Vienna, Austria; Univ Erlangen Nurnberg, Dept Dermatol, D-8520 Erlangen, Germany. FASEB JOURNAL (JUL 2001) Vol. 15, No. 9, pp. U120-U145. Publisher: FEDERATION AMER SOC EXP BIOL. 9650 ROCKVILLE PIKE, BETHESDA, MD 20814-3998 USA. ISSN: 0892-6638. Pub. country: Austria; Germany. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS
AB Worldwide more than 200 million individuals are allergic to group 1

grass pollen **allergens**. We have used the major timothy grass pollen **allergen** Phl p 1, which cross-reacts with most grass-, corn-, and monocot-derived group 1 **allergens** to develop a generally applicable strategy for the production of hypoallergenic allergy vaccines. On the basis of the experimentally determined **B cell epitopes** of Phl p 1, we have synthesized five synthetic peptides. These peptides are derived from the major Phl p 1 **IgE** epitopes and were between 28-32 amino acids long. We demonstrate by nuclear magnetic resonance that the peptides exhibit no secondary and tertiary structure and accordingly failed to bind **IgE** antibodies from grass pollen allergic patients. The five peptides, as well as an equimolar mixture thereof, lacked allergenic activity as demonstrated by basophil histamine release and skin test experiments in grass pollen allergic patients. When used as immunogens in mice and rabbits, the peptides induced protective IgG antibodies, which recognized the complete Phl p 1 wild-type **allergen** and group 1 **allergens** from other grass species. Moreover, peptide-induced antibodies inhibited the **binding** of grass pollen allergic patients **IgE** antibodies to the wild-type **allergen**. We thus demonstrate that synthetic hypoallergenic peptides derived from **B cell epitopes** of major **allergens** represent safe vaccine candidates for the treatment of **IgE** -mediated allergies.

L30 ANSWER 11 OF 77 MEDLINE

2001156261 Document Number: 21083518. PubMed ID: 11167371. **B-cell epitopes** of the **allergen** Chi t 1.01: peptide mapping of epitopes recognized by rabbit, murine, and human antibodies. van Kampen V; Liebers V; Sander I; Chen Z; Baur X; Raulf-Heimsoth M; Falkenberg F W. (Research Institute for Occupational Medicine (BGFA), Institute at the Ruhr-University of Bochum, Germany.) ALLERGY, (2001 Feb) 56 (2) 118-25. Journal code: 7804028. ISSN: 0105-4538. Pub. country: Denmark. Language: English.

AB BACKGROUND: Chi t 1.01, a hemoglobin of the midge Chironomus thummi thummi, is a widespread environmental and occupational **allergen**. The aim of the present investigation was to identify and compare peptides involved in **B-cell epitopes** of Chi t 1.01 recognized by 15 human **IgE** sera, six murine monoclonal antibodies (mAbs), and a polyclonal rabbit antiserum. METHODS: Synthetic peptides 19-21 amino acids long covering the whole Chi t 1.01-sequence were covalently coupled to activated paper disks as well as adsorbed to wells of immunoplates and used for enzyme-linked immunosorbent assay. For fine epitope mapping, we used overlapping synthetic octapeptides with one amino-acid offset. RESULTS: Peptides containing the amino acids 13-17, 23-29, and 40-50 were recognized by three of the mAbs, while three other mAbs reacting with none of the peptides obviously recognized conformational epitopes. **Binding** sites for rabbit antibodies and for human **IgE** antibodies were scattered over the whole molecule. The peptide 80-100 seemed to comprise at least one important **IgE** epitope. Depending on the method of antigen **binding** to the solid phase, differing results were obtained. CONCLUSIONS: Several linear epitopes in Chi t 1.01 are recognized by human **IgE** antibodies, by mAbs, and by polyclonal rabbit antibodies. In addition, the results indicate the presence of conformational epitopes.

L30 ANSWER 12 OF 77 SCISEARCH COPYRIGHT 2002 ISI (R)

2002:19877 The Genuine Article (R) Number: 503CZ. **IgE** and IgG **binding** epitopes on alpha-lactalbumin and beta-lactoglobulin in cow's milk allergy. Jarvinen K M; Chatchatee P; Bardina L; Beyer K; Sampson H A (Reprint). CUNY Mt Sinai Sch Med, Dept Pediat, Div Pediat Allergy & Immunol, Box 1198, 1 Gustave L Levy Pl, New York, NY 10029 USA (Reprint); CUNY Mt Sinai Sch Med, Dept Pediat, Div Pediat Allergy & Immunol, New York, NY 10029 USA; CUNY Mt Sinai Sch Med, Jaffe Inst Food

Allergy, New York, NY 10029 USA. INTERNATIONAL ARCHIVES OF ALLERGY AND IMMUNOLOGY (OCT 2001) Vol. 126, No. 2, pp. 111-118. Publisher: KARGER. ALLSCHWILERSTRASSE 10, CH-4009 BASEL, SWITZERLAND. ISSN: 1018-2438. Pub. country: USA. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Background: Cow's milk is one of the most common causes of food allergy in the first years of life. We recently defined **IgE** and IgG **binding** epitopes for alpha (s1)-casein, a major cow's milk **allergen**, and found an association between recognition of certain epitopes and clinical symptoms of cow's milk allergy (CMA). Since alpha -lactalbumin (ALA) and beta -lactoglobulin (BLG) are suspected to be significant **allergens** in cow's milk, we sought to determine the structure of sequential epitopes recognized by **IgE** antibodies to these proteins. We further sought to assess the pattern of epitope recognition in association with the clinical outcome of CMA. Methods: According to the known amino acid sequence of ALA and BLG, 57 and 77 overlapping decapeptides (offset by two amino acids), respectively, were synthesized on a cellulose derivatized membrane. Sera from 11 patients 4-18 years of age with persistent CMA (**IgE** to cow's milk > 100 kU(A)/l) were used to identify **IgE binding** epitopes. In addition, 8 patients <3 years of age and likely to outgrow their milk allergy (**IgE** to cow's milk < 30 kU(A)/l) were used to investigate the differences in epitope recognition between patients with 'persistent' and those with 'transient' CMA. Seven patients 4-18 years of age were used for assessing the IgG **binding** regions. Results: In patients with persistent allergy, four **IgE binding** and three IgG **binding** regions were identified on ALA, and seven **IgE** and six IgG **binding** epitopes were detected on BLG. The younger patients that are likely to outgrow their allergy recognized only three of these **IgE binding** epitopes on BLG and none on ALA. Conclusions: The presence of **IgE** antibodies to multiple linear allergenic epitopes may be a marker of persistent CMA. The usefulness of **IgE binding** to distinct epitopes on whey proteins in defining the patients that would have a lifelong CMA needs to be investigated in further studies. Copyright (C) 2001 S. Karger AG, Basel.

L30 ANSWER 13 OF 77 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
2001:223644 Document No.: PREV200100223644. Monoclonal antibodies raised against the major apple **allergen**, Mal d 1, are useful tools for epitope studies. Son, Dae Yeul (1); Fahlbusch, Baerbel; Mueller, Wolf-Dieter; Petersen, Arnd; Lee, Sang Il; Vieths, Stefan. (1) Department of Pediatrics, Samsung Medical Center, 50 Ilwon Dong, Kangnam Gu, Seoul, 135-710: soncha@lycos.co.kr South Korea. Food and Agricultural Immunology, (March, 2001) Vol. 13, No. 1, pp. 39-49. print. ISSN: 0954-0105. Language: English. Summary Language: English.

AB Mal d 1, the major apple **allergen** shows strong cross-reactivity with **IgE** specific for the major birch pollen **allergen**, Bet v 1, and is responsible for birch pollen related food allergy to apple. In contrast to other food and pollen **allergens**, only a few data on the **B-cell epitopes** of Mal d 1 are available. To obtain data on the antibody **binding** epitopes of Mal d 1 and to gain insights to the structures responsible for its B-cell cross-reactivity to Bet v 1, the **binding** characteristics were studied with 3 monoclonal antibodies (MAbs) raised against Mal d 1 and with patients' **IgE** directed against Mal d 1 and Bet v 1 by immunoblotting and ELISA. The different **binding** characteristics of these three MAbs indicated that the MAbs 1D6, 2B2 and 3F8 recognized different epitopes on the major apple **allergen**. MAb 2B2 cross-reacted with Bet v 1 on immunoblots, whereas 1D6 and 3F8 did not. Since 1D6 and 2B2 (but not 3F8) always competed with **IgE** from the same apple-allergic patients, 1D6 may be a useful tool for Mal d 1 epitope studies and 2B2 for an epitope that cross-reacts with **IgE**

specific for Bet v 1.

L30 ANSWER 14 OF 77 MEDLINE
2002014530 Document Number: 21312417. PubMed ID: 11419725. What establishes a protein as an **allergen**?. Bredehorst R; David K. (Institute of Biochemistry and Food Chemistry, University of Hamburg, Germany.. rbredehorst@chemie.uni-hamburg.de) . JOURNAL OF CHROMATOGRAPHY. B, BIOMEDICAL SCIENCES AND APPLICATIONS, (2001 May 25) 756 (1-2) 33-40. Ref: 83. Journal code: 9714109. ISSN: 1387-2273. Pub. country: Netherlands. Language: English.

AB There is little known about the factors that determine the allergenicity of food proteins. Apparently, the ability of a food protein to induce an allergic response requires its presence in substantial amounts in the food supply, its durability during food processing, and its resistance to digestion in the gastrointestinal tract. In addition to the mode and degree of exposure, structural characteristics appear to play an important role for the capacity of a protein to modulate the immune response towards allergic reactions. Until now, however, there has been no indication for common structural characteristics of linear T cell or linear **IgE** (**B cell**) **epitopes** and the knowledge of structural characteristics of conformational **IgE binding** sites is very limited. Experimental data point only to certain surface areas of allergenic proteins which are important for **IgE binding**. Therefore, it is not possible to suggest any structural motif or conformational sequence pattern common to all allergenic proteins. Furthermore, glycosylation appears not to be a common critical determinant of allergenicity since food **allergens** comprise both glycoproteins and nonglycosylated proteins. Based on the few published three-dimensional structures of allergenic proteins including food proteins, one unifying feature of **allergens** appears to be their spherical shape. The three-dimensional structures of many more **allergens** have to be determined, however, to allow for a better understanding of the molecular basis of allergenicity. Most recently, new ideas have been introduced as to why certain biochemical or biologic functions such as enzymatic activities may predispose a protein to become an **allergen**. Proteolytically active **allergens** have been demonstrated to irritate the human mucosal surface, to enhance their own transmucosal uptake, and to augment **IgE** production. Therefore, the functional activity of some **allergens** may play a role among other factors in the process of sensitization and allergic responses.

L30 ANSWER 15 OF 77 MEDLINE
2001086778 Document Number: 20558236. PubMed ID: 11106410. Hev b 9, an enolase and a new cross-reactive **allergen** from hevea latex and molds. Purification, characterization, cloning and expression. Wagner S; Breiteneder H; Simon-Nobbe B; Susani M; Krebitz M; Niggemann B; Brehler R; Scheiner O; Hoffmann-Sommergruber K. (Department of Pathophysiology (formerly General and Experimental Pathology), University of Vienna, Austria.) EUROPEAN JOURNAL OF BIOCHEMISTRY, (2000 Dec) 267 (24) 7006-14. Journal code: 0107600. ISSN: 0014-2956. Pub. country: GERMANY: Germany, Federal Republic of. Language: English.

AB Natural rubber latex allergy is an **IgE**-mediated disease that is caused by proteins that elute from commercial latex products. A complementary DNA (cDNA) coding for Hev b 9, an enolase (2-phospho-D-glycerate hydrolyase) and **allergen** from latex of the rubber tree Hevea brasiliensis, was amplified by PCR. The PCR primers were designed according to conserved regions of enolases from plants. The obtained cDNA amplification product consisted of 1651 bp and encoded a protein of 445 amino-acid residues with a calculated molecular mass of 47.6 kDa. Sequence comparisons revealed high similarities of the Hevea latex enolase to mold enolases that have been identified as important **allergens**. In addition, the crucial amino-acid residues that

participate in the formation of the catalytic site and the Mg2+ **binding** site of enolases were also conserved. Hevea latex enolase was produced as a recombinant protein in Escherichia coli with an N-terminal hexahistidyl tag, and purified by affinity chromatography. The yield amounted to 110 mg of purified Hev b 9 per litre of bacterial culture. The recombinant **allergen** bound **IgE** from latex, as well as mold-allergic patients, in immunoblot and ELISA experiments. The natural enolase was isolated from Hevea latex by (NH4)2SO4 precipitation and ion exchange chromatography. The natural and the recombinant (r)Hev b 9 showed equivalent enzymatic activity. Patients' **IgE**-antibodies preincubated with rHev b 9 lost their ability to bind to natural (n) Hev b 9, indicating the identity of the **B-cell epitopes** on both molecules. Cross-reactivity with two enolases from Cladosporium herbarum and Alternaria alternata was determined by inhibition of **IgE-binding** to these enolases by rHev b 9. Therefore, enolases may represent another class of highly conserved enzymes with allergenic potentials.

L30 ANSWER 16 OF 77 MEDLINE

2000420411 Document Number: 20336613. PubMed ID: 10877820. Rapid production of the major birch pollen **allergen** Bet v 1 in Nicotiana benthamiana plants and its immunological in vitro and in vivo characterization. Krebitz M; Wiedermann U; Essl D; Steinkellner H; Wagner B; Turpen T H; Ebner C; Scheiner O; Breiteneder H. (Department of Pathophysiology, University of Vienna, Austria.) FASEB JOURNAL, (2000 Jul) 14 (10) 1279-88. Journal code: 8804484. ISSN: 0892-6638. Pub. country: United States. Language: English.

AB Type I allergies are immunological disorders that afflict a quarter of the world's population. Improved diagnosis of allergic diseases and the formulation of new therapeutic approaches are based on the use of recombinant **allergens**. We describe here for the first time the application of a rapid plant-based expression system for a plant-derived **allergen** and its immunological characterization. We expressed our model **allergen** Bet v 1, the major birch pollen **allergen** , in the tobacco-related species Nicotiana benthamiana using a tobacco mosaic virus vector. Two weeks postinoculation, plants infected with recombinant viral RNA containing the Bet v 1 coding sequence accumulated the **allergen** to levels of 200 microg/g leaf material. Total nonpurified protein extracts from plants were used for immunological characterizations. **IgE** immunoblots and ELISA (enzyme-linked immunoassay) inhibition assays showed comparable **IgE** **binding** properties for tobacco recombinant (r) Bet v 1 and natural (n) Bet v 1, suggesting that the **B cell** **epitopes** were preserved when the **allergen** was expressed in N. benthamiana plants. Using a murine model of type I allergy, mice immunized with crude leaf extracts containing Bet v 1 with purified rBet v 1 produced in E. coli or with birch pollen extract generated comparable **allergen**-specific **IgE** and IgG1 antibody responses and positive type I skin test reactions. These results demonstrate that nonpurified Bet v 1 overexpressed in N. benthamina has the same immunogenicity as purified Bet v 1 produced in E. coli or nBet v 1. We therefore conclude that this plant expression system offers a viable alternative to fermentation-based production of **allergens** in bacteria or yeasts. In addition, there may be a broad utility of this system for the development of new and low-cost vaccination strategies against allergy.

L30 ANSWER 17 OF 77 MEDLINE

2000237139 Document Number: 20237139. PubMed ID: 10772962. Antibody **binding** of deletion mutants of Asp f 2, the major Aspergillus fumigatus **allergen**. Tang B; Banerjee B; Greenberger P A; Fink J N; Kelly K J; Kurup V P. (Department of Pediatrics, Medical College of Wisconsin, Milwaukee, Wisconsin, USA.) BIOCHEMICAL AND BIOPHYSICAL

RESEARCH COMMUNICATIONS, (2000 Apr 21) 270 (3) 1128-35. Journal code: 0372516. ISSN: 0006-291X. Pub. country: United States. Language: English.

AB Asp f 2, a 268 amino acid major **allergen** from Aspergillus fumigatus (Af) demonstrated nine linear **IgE binding** regions. It is not known whether any of these linear epitopes are also conformatory epitopes. Hence, we constructed deletion mutants of Asp f 2 devoid of one or more epitopes, and the **IgE binding** of these proteins with sera from patients with ABPA was compared with the full-length Asp f 2 expressed in E. coli and Pichia. The Pichia expressed protein reacted weakly with **IgE**, but strongly with IgG of ABPA sera compared to E. coli expressed Asp f 2. Weak **IgE binding** only was seen when the C-terminal or N-terminal was deleted, while depletion of both ends negated all reactivity. The monoclonal antibody IL-B8 and **IgE** and IgG of ABPA sera bound significantly to the Asp f 2 E-4 fragment indicating that the major **B-cell epitope** is located at the N-terminal end of Asp f 2.

L30 ANSWER 18 OF 77 MEDLINE

2001069742 Document Number: 20534817. PubMed ID: 11080711. **IgE-binding** epitopes of enolases, a class of highly conserved fungal **allergens**. Simon-Nobbe B; Probst G; Kajava A V; Oberkofler H; Susani M; Crameri R; Ferreira F; Ebner C; Breitenbach M. (Institute of Genetics and General Biology, University of Salzburg, Salzburg, Austria.) JOURNAL OF ALLERGY AND CLINICAL IMMUNOLOGY, (2000 Nov) 106 (5) 887-95. Journal code: 1275002. ISSN: 0091-6749. Pub. country: United States. Language: English.

AB BACKGROUND: Cladosporium herbarum and Alternaria alternata are two of the most prominent fungal species inducing type I allergy. Previously, we have demonstrated that enolase (Cla h 6) is the second most important **allergen** of C herbarum in terms of frequency of sensitization. OBJECTIVE: **IgE**-reactive **B-cell epitopes** of C herbarum enolase were analyzed, and cross-reactivity between fungal enolases was investigated. METHODS: Cla h 6 glutathione-S-transferase fusion peptides were constructed by means of PCR cloning. A alternata enolase (Alt a 5) was isolated by screening a complementary (c)DNA expression library with a C herbarum enolase DNA probe. RESULTS: Mapping of Cla h 6 **IgE-binding** epitopes identified a peptide with a length of 69 amino acids (peptide 9), which bound **IgE** from 8 of 8 patients. Analysis of the conformation of peptide 9 revealed that it does not form a compact structure but rather spans the whole length of the protein, with side chains exposed to solvent at 3 locations. Peptide 9 in the context of Escherichia coli glutathione-S-transferase not only binds **IgE** but also competitively inhibits **IgE binding** to Alt a 5. This result indicates that the epitope or epitopes on peptide 9 constitute a major cross-reacting epitope or epitopes on the enolases from C herbarum and A alternata in the case of the one patient tested. CONCLUSIONS: We demonstrated that the glycolytic enzyme enolase is an **allergen** not only in C herbarum but also in A alternata. Additionally, enolase was shown to exhibit high cross-reactivity to other fungal enolases. On the basis of the results presented here, we propose the use of recombinant Cla h 6 or maybe even peptide 9 of Cla h 6 for diagnosis and possibly therapy of mold allergy.

L30 ANSWER 19 OF 77 CAPLUS

2000:894840 Document No. 135:75643 Synthesis of glycopeptide modified **IgE** epitopes. Jablonkai, Istvan; Toth, Istvan (Dept. of Pharmaceutical and Biological Chemistry, The School of Pharmacy, University of London, London, WC1N 1AX, UK). Peptides for the New Millennium, Proceedings of the American Peptide Symposium, 16th, Minneapolis, MN, United States, June 26-July 1, 1999, Meeting Date 1999,

775-776. Editor(s): Fields, Gregg B.; Tam, James P.; Barany, George. Kluwer Academic Publishers: Dordrecht, Neth. (English) 2000. CODEN: 69ATHX.

AB **Allergen**-specific **IgE** activates the mast cells to release histamine, heparin and other mediators responsible for the allergic reactions. One of the ways to initiate an immune response against non-peptidic antigens (saccharides) is to conjugate them with adjuvant/carriers. As model compds. for the synthesis of sugar antigen/**B-cell epitope** complexes, some glycopeptide modified **IgE-binding** epitopes of **allergen** proteins from peanut Ara h1 and house dust mite are described.

L30 ANSWER 20 OF 77 SCISEARCH COPYRIGHT 2002 ISI (R)

2000:153581 The Genuine Article (R) Number: 285RQ. Mutational analysis of the **IgE-binding** epitopes of P34/Gly m Bd 30K. Helm R M (Reprint); Cockrell G; Connaughton C; West C M; Herman E; Sampson H A; Bannon G A; Burks A W. UNIV ARKANSAS MED SCI, ARKANSAS CHILDRENS NUTR CTR, DEPT PEDIAT, 1120 MARSHALL ST, LITTLE ROCK, AR 72202 (Reprint); UNIV ARKANSAS MED SCI, DEPT BIOCHEM & MOL BIOL, LITTLE ROCK, AR 72202; AGR RES SERV, CLIMATE STRESS LAB, USDA, BELTSVILLE, MD; MT SINAI SCH MED, DIV PEDIAT ALLERGY & IMMUNOL, NEW YORK, NY. JOURNAL OF ALLERGY AND CLINICAL IMMUNOLOGY (FEB 2000) Vol. 105, No. 2, Part 1, pp. 378-384. Publisher: MOSBY-YEAR BOOK INC. 11830 WESTLINE INDUSTRIAL DR, ST LOUIS, MO 63146-3318 . ISSN: 0091-6749. Pub. country: USA. Language: English.

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Background: Peanuts and soybeans are 2 foods that have been shown to be responsible for many atopic disorders. Because of their nutritional benefit, soybean proteins are now being used increasingly in a number of food products. Previous studies have documented multiple **allergens** in soybean extracts, including glycinin, beta-conglycinin, and the P34/Gly m Bd 30K protein.

Objective: Our overall goal was to identify soybean-specific **allergens** to begin to understand molecular and immunochemical characteristics of legume proteins. The specific aim of the current investigation was to identify the essential amino acid residues necessary for **IgE binding** in the 5 distinct immunodominant epitopes of P34/Gly m Ed 30K,

Methods: Serum **IgE** from 6 clinically sensitive soybean-allergic individuals was used to identify P34/Gly m Ed 30K in the native and single amino acid substituted peptides with use of the SPOTS peptide synthesis technique to determine critical amino acids required for **IgE binding**.

Results: The intensity of **IgE binding** and epitope recognition by serum **IgE** from the individuals varied substantially. With use of serum from 6 clinically soybean-sensitive individuals, 2 of the 5 immunodominant epitopes could be mutagenized to non-**IgE binding** peptides,

Conclusions: Single-site amino acid substitution of the 5 immunodominant epitopes of Gly m Ed 30K with alanine revealed that **IgE binding** could be reduced or eliminated in epitopes 6 and 16 in the serum obtained from 6 soybean-sensitive patients.

L30 ANSWER 21 OF 77 MEDLINE

2000318733 Document Number: 20318733. PubMed ID: 10861069. Dominant epitopes and allergic cross-reactivity: complex formation between a Fab fragment of a monoclonal murine IgG antibody and the major **allergen** from birch pollen Bet v 1. Mirza O; Henriksen A; Ipsen H; Larsen J N; Wissenbach M; Spangfort M D; Gajhede M. (Protein Structure Group, Department of Chemistry, University of Copenhagen, Denmark.) JOURNAL OF IMMUNOLOGY, (2000 Jul 1) 165 (1) 331-8. Journal code: 2985117R. ISSN: 0022-1767. Pub. country: United States. Language: English.

AB The symptoms characteristic of allergic hypersensitivity are caused by the release of mediators, i.e., histamine, from effector cells such as

basophils and mast cells. **Allergens** with more than one **B cell epitope** cross-link **IgE** Abs bound to high affinity FcepsilonRI receptors on mast cell surfaces leading to aggregation and subsequent mediator release. Thus, **allergen**-Ab complexes play a crucial role in the cascade leading to the allergic response. We here report the structure of a 1:1 complex between the major birch pollen **allergen** Bet v 1 and the Fab fragment from a murine monoclonal IgG1 Ab, BV16, that has been solved to 2.9 A resolution by x-ray diffraction. The mAb is shown to inhibit the **binding** of allergic patients' **IgE** to Bet v 1, and the **allergen**-IgG complex may therefore serve as a model for the study of **allergen-IgE** interactions relevant in allergy. The size of the BV16 epitope is 931 A2 as defined by the Bet v 1 Ab interaction surface. Molecular interactions predicted to occur in the interface are likewise in agreement with earlier observations on Ag-Ab complexes. The epitope is formed by amino acids that are conserved among major **allergens** from related species within the Fagales order. In combination with a surprisingly high inhibitory capacity of BV16 with respect to allergic patients' serum **IgE binding** to Bet v 1, these observations provide experimental support for the proposal of dominant **IgE** epitopes located in the conserved surface areas. This model will facilitate the development of new and safer vaccines for **allergen** immunotherapy in the form of mutated **allergens**.

L30 ANSWER 22 OF 77 MEDLINE

2000290931 Document Number: 20290931. PubMed ID: 10828716. Regulation of specific immune responses by chemical and structural modifications of **allergens**. Akdis C A; Blaser K. (Swiss Institute of Allergy and Asthma Research (SIAF), Davos, Switzerland.. akdisac@siaf.unizh.ch) . INTERNATIONAL ARCHIVES OF ALLERGY AND IMMUNOLOGY, (2000 Apr) 121 (4) 261-9. Ref: 103. Journal code: 9211652. ISSN: 1018-2438. Pub. country: Switzerland. Language: English.

AB Specific immunotherapy (SIT) is an efficient treatment of allergic diseases to defined **allergens**. Despite being used in clinical practice since early in this century, more rational and safer regimens are required, because SIT is faced with the risk of anaphylaxis and standardization problems of **allergen**-extract-based treatments. A better understanding of the pathogenesis of allergy and of the mechanisms of SIT has led to various approaches to overcome these problems. Knowledge of the influence of **IgE**-facilitated antigen presentation on **allergen**-specific Th2 responses increased the efforts to generate non-**IgE-binding allergens**. The current principal approach to **allergen** modification is to modify **B cell epitopes** in order to prevent **IgE binding** and effector cell cross-linking while preserving T cell epitopes to retain the capacity of inducing tolerance. In this way, the modified **allergen** will be directed to T cells by a phagocytosis/pinocytosis-mediated antigen uptake mechanism, bypassing **IgE** cross-linking and **IgE**-dependent antigen presentation. Accordingly, a differential regulation of **allergen**-specific T cell cytokine patterns and **IgE**:IgG production was demonstrated by modifications of the three-dimensional structure of **allergens** because of linearity in T cell epitopes and conformation dependence in **B cell epitopes**. In this context, chemically modified **allergen** extracts with low **IgE-binding** capacity have been developed to reduce anaphylactic side effects since the early 1980s. The progress of recombinant techniques for producing **allergens** and **allergen** derivatives has led to a dramatic improvement in the ability of developing novel vaccines for the treatment of allergy. This has enabled mutation or deletion of decisive amino acids in **B cell epitopes** and fractionation or oligomerization of **allergens** by genetic engineering as fruitful approaches to

generate hypoallergenic vaccines. Moreover, non-**IgE**-**binding** short T cell epitope peptides and single-amino-acid-altered peptide ligands represent potential candidates for future SIT. Copyright 2000 S. Karger AG, Basel

L30 ANSWER 23 OF 77 MEDLINE

2001086250 Document Number: 20564015. PubMed ID: 11112859. Identification of a sequential **B-cell epitope** on major **allergen** (Cry j 1) of Japanese cedar (Cryptomeria japonica) pollen in mice. Tamura Y; Sasaki R; Inouye S; Kawaguchi J; Serizawa N; Toda M; Takemori T; Sakaguchi M. (Department of Immunology, National Institute of Infectious Diseases, Sankyo Co., Tokyo, Japan.. msakaguc@nih.go.jp) . INTERNATIONAL ARCHIVES OF ALLERGY AND IMMUNOLOGY, (2000 Nov) 123 (3) 228-35. Journal code: 9211652. ISSN: 1018-2438. Pub. country: Switzerland. Language: English.

AB BACKGROUND: Japanese cedar (Cryptomeria japonica; CJ) pollinosis is one of the most common allergic diseases in Japan. **B cell epitopes** on Cry j 1, a major **allergen** of CJ pollen, have been analyzed by the specific monoclonal antibodies to Cry j 1, and most of these epitopes may be conformational, but no previous report has addressed the analysis of sequential epitope mapping with synthetic peptides. The main purpose of the present study is to identify **IgE** and IgG **B cell epitopes** on Cry j 1 by using a synthetic peptide approach in mice. METHODS: We synthesized 35 overlapping peptides that cover the entire length of Cry j 1 and examined whether mouse **IgE** and IgG antibodies produced by immunization with Cry j 1 reacted to the Cry j 1 peptides. RESULTS AND CONCLUSION: We found that mouse **IgE** and IgG antibodies reacted strongly to Cry j 1 peptide No. 15 ((141)GVEPVHPQDGDALTLRTATN(160)), though those antibodies did not react with other peptides. **IgE** and IgG antibody **binding** to peptide No. 15 was completely inhibited by Cry j 1 and the peptide. To determine the minimum epitope in peptide No. 15, we conducted an ELISA inhibition test. **IgE** and IgG antibody **binding** to peptide No. 15 was inhibited by smaller peptides of this peptide. We found the core of the epitope to be (145)VHPQDGDA(152). Copyright 2000 S. Karger AG, Basel

L30 ANSWER 24 OF 77 MEDLINE

2001086248 Document Number: 20564013. PubMed ID: 11112857. A soybean G2 glycinin **allergen**. 2. Epitope mapping and three-dimensional modeling. Helm R M; Cockrell G; Connaughton C; Sampson H A; Bannon G A; Beilinson V; Nielsen N C; Burks A W. (Department of Pediatrics, University of Arkansas for Medical Sciences, Arkansas Children's Nutrition Center, Little Rock, AR 72202-3591, USA.) INTERNATIONAL ARCHIVES OF ALLERGY AND IMMUNOLOGY, (2000 Nov) 123 (3) 213-9. Journal code: 9211652. ISSN: 1018-2438. Pub. country: Switzerland. Language: English.

AB BACKGROUND: Multiple **allergens** have been documented in soybean extracts. **IgE** from individuals allergic to soybeans, but not to peanut, has been shown by immunoblot analysis to bind to proteins with a molecular weight of approximately 22 kD. These findings suggested that this unique protein fraction from soybean might be responsible, in part, for soybean allergic reactivity. The objective of the present study was to characterize specific **B cell epitopes**, to determine if any amino acid was critical to **IgE binding** and to model the 22-kD G2 soybean **allergen** to the three-dimensional (3-D) phaseolin molecule. METHODS: **B cell epitopes** were identified using SPOTs peptide analysis. Structural orientation of the **IgE-binding** regions was mapped to the 3-D phaseolin molecule using molecular modeling of the protein tertiary structure. RESULTS: Eleven linear epitopes, representing 15 amino acid peptide sequences, bound to **IgE** in the glycinin molecule. These epitopes were predicted to be distributed asymmetrically on the surface of G2 trimers. CONCLUSIONS: Only 1 epitope

could be rendered non-**IgE binding** by alanine substitutions in the peptide. The nonrandom distribution of the **IgE binding** sites provides new insight into their organization in trimers in 11S complexes of the G2 glycinin **allergen**.
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L30 ANSWER 25 OF 77 SCISEARCH COPYRIGHT 2002 ISI (R)
2000:98117 The Genuine Article (R) Number: 279EH. Reduced immunogenicity of beta-lactoglobulin by conjugation with carboxymethyl dextran. Hattori M (Reprint); Nagasawa K; Ohgata K; Sone N; Fukuda A; Matsuda H; Takahashi K. TOKYO UNIV AGR & TECHNOL, FAC AGR, DEPT APPL BIOL SCI, 3-5-8 SAIWAI CHO, FUCHU, TOKYO 1838509, JAPAN (Reprint); TOKYO UNIV AGR & TECHNOL, FAC AGR, DEPT VET CLIN, FUCHU, TOKYO 1838509, JAPAN. BIOCONJUGATE CHEMISTRY (JAN-FEB 2000) Vol. 11, No. 1, pp. 84-93. Publisher: AMER CHEMICAL SOC. 1155 16TH ST, NW, WASHINGTON, DC 20036. ISSN: 1043-1802. Pub. country: JAPAN. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS
AB We prepared two beta-lactoglobulin (beta-LG)-carboxymethyl dextran (CMD) conjugates (Conj. 10A and Conj. 10B) by using a water-soluble carbodiimide to decrease the immunogenicity of beta-LG, The molar ratios of beta-LG to CMD in the conjugates were 5:1 (Conj. 10A) and 2:1 (Conj. 10B). The beta-LG-CMD conjugates maintained the retinol-**binding** activity of native beta-LG. Intrinsic fluorescence study indicated that shielding of the surface of beta-LG by CMD occurred in each conjugate, which was eminent in Conj. 10B. A local conformational change around (125)Thr-(135)Lys (alpha-helix) in each conjugate was detected by ELISA with monoclonal antibodies. The denaturation temperature of beta-LG evaluated by differential scanning calorimetry was greatly enhanced in each conjugate. The anti-beta-LG antibody response was markedly reduced after immunization with the beta-LG-CMD conjugates in BALB/c, C57BL/6,and C3H/He mice. We determined the **B cell epitopes** of beta-LG and each conjugate recognized in these mice and found that the linear epitope profiles of the beta-LG-CMD conjugates were similar to those of beta-LG, while the antibody response for each epitope was dramatically reduced. The reduced immunogenicity of beta-LG was most marked in the case of Conj. 10B, which contained more CMD than Conj. 10A, and was effectively shielded by CMD. We concluded that masking of epitopes by CMD is responsible for the decreased immunogenicity of the beta-LG in these conjugates.

L30 ANSWER 26 OF 77 CAPLUS COPYRIGHT 2002 ACS
1999:614141 Document No. 131:241995 Mutant recombinant **allergens** for use as allergy vaccines. Ipsen, Hans Henrik; Spangfort, Michael Dho; Larsen, Jorgen Nedergaard (Alk-Abello A/S, Den.). PCT Int. Appl. WO 9947680 A1 19990923, 77 pp. DESIGNATED STATES: W: AE, AL, AM, AT, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, CZ, DE, DE, DK, DK, EE, EE, ES, FI, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1999-DK136 19990316. PRIORITY: DK 1998-364 19980316.
AB Novel recombinant **allergens** are disclosed. The **allergens** are non-naturally occurring mutants derived from naturally-occurring **allergens**. The overall .alpha.-carbon backbone tertiary structure is essentially preserved. Also disclosed are methods for prepg. such recombinant **allergens** as well as uses thereof. The invention is based on the idea that the mechanism of successful allergy vaccination is not an alteration of the ongoing Th2-type immune response, but rather a parallel initiation of a new Th1-type immune response involving tertiary epitope recognition by B-cells

and antibody formation. Addnl., dominant **IgE binding** epitopes are proposed. These epitopes are supposed to be constituted by tertiary structure dependent coherent surface areas large enough to accommodate antibody **binding** and conserved among isoallergens, variants, and/or homologous **allergens** from related species. Mutant forms of Bet v 1 and Ves v 5 **allergens** were produced. The Bet v 1 mutants displayed reduced **IgE binding** although the tertiary structure of the wild-type Bet v 1 **allergen** was retained. A "triple-patch mutant" of Bet v 1 was able to induce proliferation in T cell lines from 3 different birch pollen allergic patients with stimulation indexes similar to recombinant and naturally occurring Bet v 1.

L30 ANSWER 27 OF 77 CAPLUS COPYRIGHT 2002 ACS
1999:311109 Document No. 130:351217 Tolerogenic fragments of natural **allergens**. Berrens, Lubertus; Gonzales Romano, Maria Leticia; Gallego Camara, Maria Teresa (C.B.F. Leti, S.A., Spain). PCT Int. Appl. WO 9922762 A1 19990514, 36 pp. DESIGNATED STATES: W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1997-NL598 19971030.

AB The present invention relates to a process for the controlled enzymic cleavage of purified and depigmented active allergenic proteins, which process produces fragments of **allergens** that retain the natural T-lymphocyte stimulating epitopes, but are depleted of **IgE-binding B-cell epitopes** and complement-activating agents. The allergenic proteins are derived from indoor and outdoor source materials such as insects, mites, molds, pollen of grasses, weeds, flowers, shrubs and trees, and org. dusts in occupational environments. These **allergen** fragments do not exhibit the disadvantages of conventional allergenic exts. for immunotherapy and can be safely used to induce a state of specific T-cell anergy and immunol. tolerance in allergic human beings.

L30 ANSWER 28 OF 77 MEDLINE
1999242810 Document Number: 99242810. PubMed ID: 10225885. Conformational and linear **B-cell epitopes** of Asp f 2, a major **allergen** of Aspergillus fumigatus, bind differently to immunoglobulin E antibody in the sera of allergic bronchopulmonary aspergillosis patients. Banerjee B; Greenberger P A; Fink J N; Kurup V P. (Department of Medicine, Allergy-Immunology Division, Medical College of Wisconsin, Milwaukee, Wisconsin 53226, USA.) INFECTION AND IMMUNITY, (1999 May) 67 (5) 2284-91. Journal code: 0246127. ISSN: 0019-9567. Pub. country: United States. Language: English.

AB Asp f 2 is a major Aspergillus fumigatus **allergen** involved in allergic bronchopulmonary aspergillosis. Knowledge of the **B-cell epitopes** may contribute to the understanding of immunoregulation and immunodiagnosis. To elucidate the immunoglobulin E (**IgE**) **binding** epitopes in the linear sequence of Asp f 2, we synthesized decamer peptides spanning the whole molecule of Asp f 2 on derivatized cellulose membranes and evaluated **IgE binding** in ABPA patient and control sera. Peptides three to five amino acids long were synthesized based on amino acid sequences within the **IgE binding** regions and evaluated for the specificity of epitope antibody interactions. Nine **IgE binding** regions were recognized in this protein of 268 amino acid residues. Of the nine epitopes, seven (ATQRRQI, RKYFG, HWR, YTTRR, DHFAD, ALEAYA, and THEGGQ) are present in the hydrophilic regions of Asp f 2. Immunologic evaluation of the three recombinant fragments, Asp f 2A encompassing the

N-terminal epitope region, Asp f 2B without N- and C-terminal regions of the protein, and Asp f 2C representing C-terminal epitopes, revealed that either the N- or C-terminal region of the protein is essential for the correct folding and conformation for **IgE** antibody **binding**.

L30 ANSWER 29 OF 77 MEDLINE
1999289433 Document Number: 99289433. PubMed ID: 10359901. Human **IgE-binding** epitopes of the latex **allergen** Hev b 5. Beezhold D H; Hickey V L; Slater J E; Sussman G L. (Laboratory of Immunobiology, Guthrie Research Institute, Sayre, PA 18840, USA.) JOURNAL OF ALLERGY AND CLINICAL IMMUNOLOGY, (1999 Jun) 103 (6) 1166-72. Journal code: 1275002. ISSN: 0091-6749. Pub. country: United States. Language: English.

AB BACKGROUND: Hev b 5 is an acidic protein (isoelectric point, 3.5) rich in glutamic acid with 9 repeated amino acid (AA) sequences of XEEX or XEEEX. Although its function in Hevea brasiliensis is unknown, Hev b 5 has been identified as a major latex **allergen**. Immunoblot inhibition studies suggest Hev b 5 exists as multiple isoforms or contains a common epitope found in several other proteins. OBJECTIVE: The purpose of this study was to further characterize Hev b 5 and to identify linear **IgE-binding** epitopes. METHODS: Octapeptides spanning the entire Hev b 5 protein were synthesized on a derivatized cellulose membrane. The membrane was reacted with sera pooled from health care workers allergic to latex or rabbits immunized with latex proteins. **B-cell epitopes** were identified by subsequent incubations with the appropriate secondary antibodies and detected by using chemifluorescence. RESULTS: Sera from patients allergic to latex recognized 6 **IgE-binding** regions located throughout the molecule. Two epitopes (2 and 4) had the common AA sequence of KTEEP. Epitopes 3 and 5 had a similar AA sequence of EEXXA, where X was P, T, or K. Epitopes 1 and 6 appeared to be unrelated to the other epitopes. Database analysis could not identify other proteins with similar sequences. Neither of the XEEEX sequences bound **IgE**. Control sera failed to react to any peptides. CONCLUSIONS: Hev b 5 exists as multiple isoforms, but only small amounts are present in the nonammoniated latex preparations, such as those used for diagnostic tests, and this may help to explain the relatively poor sensitivity of some in vitro tests.

L30 ANSWER 30 OF 77 SCISEARCH COPYRIGHT 2002 ISI (R)
1999:793486 The Genuine Article (R) Number: 245JV. Characterization of a dodecapeptide containing a dominant epitope of Par j 1 and Par o 1, the major **allergens** of P-judaica and P-officinalis pollen. Menna T; Cassese G; DiModugno F; Chersi A; Buono C; Ruffilli A (Reprint). CNR, INT INST GENET & BIOPHYS, VIA G MARCONI 10, I-80125 NAPLES, ITALY (Reprint); CNR, INT INST GENET & BIOPHYS, I-80125 NAPLES, ITALY; REGINA ELENA INST CANC RES, I-00161 ROME, ITALY. ALLERGY (OCT 1999) Vol. 54, No. 10, pp. 1048-1057. Publisher: MUNKSGAARD INT PUBL LTD. 35 NORRE SOGADE, PO BOX 2148, DK-1016 COPENHAGEN, DENMARK. ISSN: 0105-4538. Pub. country: ITALY. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The pollen of Parietaria, a weed of the Urticaceae family, is a major cause of respiratory allergy in Europe, where the most common species are P.judaica and P. officinalis. Previously, we reported that a beta-galactosidase fusion protein (Ga-BG) expressing a 26-bp cDNA fragment (6a cDNA) contained a dominant **IgE-binding** epitope (6a epitope) of the major **allergens** Par J 1 and Par J 1. The present study aimed to define. the amino-acid sequence containing the 6a epitope. We analyzed the reactivity of anti-Par o 1 antibodies affinity purified from allergic patient sera with:

1) a panel of synthetic peptides deduced from the 6a nucleotide sequence using different reading frames

2) glutathione 5-transferase (GST) fusion proteins containing selected

peptides.

The peptide NSARARADSCRI (p102) specifically bound anti-Par o 1 antibodies affinity purified from allergic patient sera or from rabbit anti-Par o 1 antiserum (ELISA). The related peptide NSARAGTSSCRI (p101) reacted to human but not to rabbit, anti-Par o 1 antibodies. GST fusion proteins containing p101 (GST 3.5) or p102 (GST 3.2) extensively inhibited the **binding** between Par o 1 and **IgE** or IgG antibodies from an allergic patient serum pool according to a dose-response curve. Percent inhibition of: **IgE** antibodies **binding** obtained by absorbing a solution (50 pi) of affinity-purified antibodies with 5 mu g of GST 3.2 or with 1.2 mg of GST 3.5 was 69% and 66%, respectively. In conclusion, the results of the present study indicate that the amino-acid sequences NSARARADSCRI (p102) and NSARAGTSSCRI (p101) contain the dominant epitope of Par o I and Par J 2 for human **IgE** and IgG antibodies indicated as 6a epitope. Moreover, the study shows that the epitope is conserved in recombinant molecules containing these peptides, irrespective of the fused polypeptide (beta-galactosidase or GST). The knowledge of the amino-acid sequence of this dominant epitope is important in therapeutic approaches to the development of **allergen**-derived haptens.

L30 ANSWER 31 OF 77 MEDLINE

1999268969 Document Number: 99268969. PubMed ID: 10336602. Molecular characterization of Dau c 1, the Bet v 1 homologous protein from carrot and its cross-reactivity with Bet v 1 and Api g 1. Hoffmann-Sommergruber K; O'Riordain G; Ahorn H; Ebner C; Laimer Da Camara Machado M; Puhringer H; Scheiner O; Breiteneder H. (Department of General and Experimental Pathology, University of Vienna, Austria.) CLINICAL AND EXPERIMENTAL ALLERGY, (1999 Jun) 29 (6) 840-7. Journal code: 8906443. ISSN: 0954-7894. Pub. country: ENGLAND: United Kingdom. Language: English.

AB BACKGROUND: Up to 70% of patients with birch pollen allergy exhibit the so-called oral allergy syndrome, an **IgE**-mediated food allergy. The most frequent and therefore best characterized pollen-fruit syndrome is apple allergy in patients suffering from tree pollen-induced pollinosis. The occurrence of adverse reactions to proteins present in vegetables such as celery and carrots in patients suffering from pollen allergy has also been reported. cDNAs for Bet v 1 homologous proteins have been cloned from celery, apple and cherry. Objective The aim of the study was to identify Bet v 1 homologues from carrot (Daucus carota), to isolate the respective cDNA, to compare the **IgE-binding** capacity of the natural protein to the recombinant **allergen** and determine the cross-reactivity to Api g 1 and Bet v 1. METHODS: Molecular characterization of the carrot **allergen** was performed using **IgE**-immunoblotting, cross-inhibition assays, N-terminal sequencing, PCR-based cDNA cloning and expression of the recombinant protein in Escherichia coli. RESULTS: A 16-kDa protein from carrot was identified as a major **IgE-binding** component and designated Dau c 1. Sequencing corresponding cDNAs revealed three extremely similar sequences (Dau c 1.1, 1.2 and 1.3) with an open reading frame of 462 bp coding for 154 amino acid residues. CONCLUSIONS: Purified recombinant Dau c 1.2 was tested in immunoblots displaying **IgE-binding** capacity comparable to its natural counterpart. Cross-inhibition assays verified the existence of common **B-cell epitopes** present on Dau c 1, Api g 1 as well as on Bet v 1.

L30 ANSWER 32 OF 77 MEDLINE

1999355637 Document Number: 99355637. PubMed ID: 10425162. Expressions of recombinant venom **allergen**, antigen 5 of yellowjacket (Vespula vulgaris) and paper wasp (Polistes annularis), in bacteria or yeast. Monsalve R I; Lu G; King T P. (The Rockefeller University, 1230 York Avenue, New York, New York 10021-6399, USA.) PROTEIN EXPRESSION AND PURIFICATION, (1999 Aug) 16 (3) 410-6. Journal code: 9101496. ISSN: 1046-5928. Pub. country: United States. Language: English.

AB Antigen 5 is a major **allergen** of vespid venom. It has partial sequence identity with proteins from diverse sources. The biologic function of Ag 5 and its related proteins is not known. We are interested in the expression of Ag 5 with the native conformation of the natural protein since its **B cell epitopes** are mainly of the discontinuous type. When expressed in bacteria, recombinant Ag 5 formed an insoluble intracellular product, and it did not translocate from cytoplasm to periplasm by the addition of a pelB leader sequence to the cloned protein. When expressed in yeast Pichia pastoris, Ag 5 was secreted because the cloned protein contained a yeast alpha signal leader sequence. Recombinant Ag 5 from yeast was shown to have the native structure of the natural protein and the recombinant Ag 5 from bacteria did not. This was shown by comparison of their solubility, electrophoretic behavior, disulfide bond content, CD spectrum, and **binding** of **IgE** antibodies from allergic patients and IgG antibodies from mice immunized with natural Ag 5 or recombinant Ag 5s from yeast or bacteria. These studies were made with Ag 5s from yellowjacket (Vespula vulgaris) and paper wasp (Polistes annularis).

L30 ANSWER 33 OF 77 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
2000:64680 Document No.: PREV200000064680. Mapping of **IgE** **binding** regions in the major rat urinary protein, alpha2u-globulin, using overlapping peptides. Bayard, C. (1); Siddique, A. B.; Berzins, K.; Troye-Blomberg, M.; Hellman, U.; Vesterberg, O.. (1) Department of Occupational Medicine, National Institute for Working Life, S-171 84, Solna Sweden. Immunological Investigations, (Sept. Dec., 1999) Vol. 28, No. 5-6, pp. 323-338. ISSN: 0882-0139. Language: English. Summary Language: English.
AB Exposure to laboratory animals poses a hazard for development of occupational allergy. Identification of antigenic determinants of allergenic proteins may be valuable for immunotherapeutic purposes. Overlapping peptides of the major **allergen** in rat urine, Rat n 1.02, corresponding to the protein alpha2u-globulin were synthesised on solid support and screened simultaneously to locate **IgE** **binding** linear epitopes using a simple modified ELISA procedure. Thirty-nine peptides were synthesised, each 8 amino acids long with 4 amino acids overlaps. Sera from fifteen rat-sensitized subjects were analyzed and as controls sera from 7 non-rat-sensitized individuals were used. In general low **binding** and a great individual variation between sera from rat allergic individuals were seen. Some peptides were more frequently recognized by **IgE** antibodies in sera from rat allergics. These peptides were mainly clustered towards the N-terminal and C-terminal parts of the protein. Taken together our data suggest the existence of linear **IgE** **binding** epitopes in the rat urine **allergen**, Rat n 1.02. However, the role of these sequences in the allergic reaction needs further investigation.

L30 ANSWER 34 OF 77 MEDLINE
2000109258 Document Number: 20109258. PubMed ID: 10640911. The recognition pattern of sequential **B cell** **epitopes** of beta-lactoglobulin does not vary with the clinical manifestations of cow's milk allergy. Heinzmann A; Blattmann S; Spuergin P; Forster J; Deichmann K A. (University Children's Hospital, University of Freiburg, Freiburg, Germany.) INTERNATIONAL ARCHIVES OF ALLERGY AND IMMUNOLOGY, (1999 Dec) 120 (4) 280-6. Journal code: 9211652. ISSN: 1018-2438. Pub. country: Switzerland. Language: English.
AB BACKGROUND: beta-Lactoglobulin (BLG) represents one of the major **allergens** causing cow's milk allergy (CMA) - a disease with a wide spectrum of clinical symptoms. The aim of this study was to evaluate sequential **B cell epitopes** of BLG by the Pin-ELISA method. Furthermore, we wanted to investigate a possible association of the **IgE** recognition patterns in sera of patients

with BLG sensitization and the type of clinical reactions following contact with cow's milk. METHODS: Overlapping sequential decapeptides corresponding to the amino acid sequence of BLG were used in Pin-ELISAs specific for human **IgE**. Tested sera were from 14 individuals with CMA, 8 of them with a history of immediate systemic reactions and 6 with delayed skin reactions following contact with cow's milk. All of them showed specific **IgE** antibodies to BLG in the CAP-RAST. Control sera were from 5 healthy nonallergic individuals. RESULTS: All sera from BLG-sensitized individuals showed **IgE binding** with one region of BLG corresponding to amino acids 95-113. Furthermore, individual sera showed reactions with two further regions, 12-27 and 124-135. Inhibition of **IgE binding** to BLG with one soluble synthetic peptide confirmed the major epitope. No differences were found in the **B cell epitope** recognition pattern to BLG in the two groups of patients with CMA, characterized by acute systemic or delayed skin reactions. CONCLUSIONS: Using **IgE** Pin-ELISAs we were able to confirm previously described sequential **B cell epitopes** of BLG. However, the recognition pattern of one of the major cow's milk **allergens** is not predictive of the clinical type of reaction.

L30 ANSWER 35 OF 77 MEDLINE

2000109255 Document Number: 20109255. PubMed ID: 10640908. Important animal **allergens** are lipocalin proteins: why are they allergenic?. Virtanen T; Zeiler T; Mantyjarvi R. (Department of Clinical Microbiology, University of Kuopio, Kuopio, Finland.. tuomas.virtanen@uku.fi) . INTERNATIONAL ARCHIVES OF ALLERGY AND IMMUNOLOGY, (1999 Dec) 120 (4) 247-58. Ref: 161. Journal code: 9211652. ISSN: 1018-2438. Pub. country: Switzerland. Language: English.

AB Major respiratory **allergens** of dogs, mice, rats, horses and cows belong to the lipocalin group of proteins. The sequence identity of lipocalins is often less than 20%, but they contain between one and three structurally conserved regions and their three-dimensional structures are similar. Lipocalins share common biological functions, predominantly related to the transport of small hydrophobic molecules, such as vitamins and pheromones. Immune reactivity to lipocalin **allergens** is not well known. In Bos d 5, the **IgE-binding** epitopes are spread along the molecule, whereas in Bos d 2, the C terminus appears to contain the human **B cell epitopes**. Bos d 5 contains several murine T cell epitopes. No information is available on human T cell epitopes. The maximal number of epitopes an allergic patient's T cells could recognize in Bos d 2 was five. Three of the epitopes were colocalized in the structurally conserved regions of lipocalins. Interestingly, one of the epitopes was recognized by the T cells of all patients and the computer predictions suggested that there would be an epitope in the corresponding parts of human endogenous lipocalins. The proliferative responses of peripheral blood mononuclear cells of Bos d 2-allergic subjects to Bos d 2 were weak. The T cell response was Th2-dominated. To explain these observations, we have proposed that the allergenicity of lipocalins may be a consequence of molecular mimicry between lipocalin **allergens** and endogenous lipocalins at the T cell level.

L30 ANSWER 36 OF 77 CAPLUS COPYRIGHT 2002 ACS

1999:349930 Document No. 131:156840 Nonapeptides selected by phage display mimic the **binding** sites of monoclonal antibodies BIP1 and BIP4 on Bet v 1, the major birch pollen **allergen**. Jensen-Jarolim, E.; Ganglberger, E.; Leitner, A.; Radauer, C.; Scheiner, O.; Breiteneder, H. (Department of General and Experimental Pathology, University of Vienna, Vienna, A-1090, Austria). International Archives of Allergy and Immunology, 118(2-4), 224-225 (English) 1999. CODEN: IAAIEG. ISSN: 1018-2438. Publisher: S. Karger AG.

AB In birch pollen allergy the major **allergen** Bet v 1 is recognized

by **IgE** from more than 90% of allergic patients. Moreover, 60% of the patients show exclusive **IgE binding** to Bet v 1. From studies with monoclonal antibodies, it is known that IgG specific for this **allergen** affects the **IgE-allergen** interaction. The authors have previously defined **B cell epitopes** of monoclonal anti-Bet v 1 antibodies BIP1 and BIP4 by using random nonapeptide phage display peptide libraries and compared the selected mimotopes with the sequence and 3D structure of Bet v 1. Anal. showed that specific ligands were selected from the constrained library. As expected, alignments with the sequence of Bet v 1 showed no homol. of the BIP1 mimotope CFPYCYPSESA and the BIP4 mimotope CRQTRTMPGC. The highest rate of similarities for the BIP1 mimotope was found between Phe and Tyr of Bet v 1. Here, alignment with the 3D structure showed that this part of the sequence composes an exposed loop between beta strands 3 and 4. The majority of amino acids of this hypothetical epitope are less accessible; the authors speculate that they may participate to **binding** by induced fit.

L30 ANSWER 37 OF 77 MEDLINE

1999077576 Document Number: 99077576. PubMed ID: 9862740. Molecular and immunologic characterization of a highly cross-reactive two EF-hand calcium-**binding** alder pollen **allergen**, Aln g 4: structural basis for calcium-modulated **IgE** recognition. Hayek B; Vangelista L; Pastore A; Sperr W R; Valent P; Vrtala S; Niederberger V; Twardosz A; Kraft D; Valenta R. (Department of General and Experimental Pathology, AKH, University of Vienna, Austria.) JOURNAL OF IMMUNOLOGY, (1998 Dec 15) 161 (12) 7031-9. Journal code: 2985117R. ISSN: 0022-1767. Pub. country: United States. Language: English.

AB Serum **IgE** was used to isolate a cDNA coding for a 9.4-kDa two EF-hand calcium-**binding allergen**, Aln g 4, from a lambda gt11 expression cDNA library constructed from alder (Alnus glutinosa) pollen. rAln g 4 was overexpressed in Escherichia coli and purified to homogeneity. It reacted with serum **IgE** from 18% of pollen-allergic patients (n = 122); shared **IgE** epitopes with homologous **allergens** present in tree, grass, and weed pollens; and thus belongs to a family of highly cross-reactive pollen **allergens**. Exposure of two E. coli-expressed rAln g 4 fragments comprising amino acids 1-41 and 42-85 to patients' **IgE** Abs, as well as to a rabbit antiserum raised against purified rAln g 4, indicated that most of the **B cell epitopes** reside in the N-terminal portion of the protein. **IgE** recognition of Aln g 4 was strongly modulated by the presence or absence of calcium. Circular dichroism analysis of rAln g 4 revealed that the protein consisted mostly of alpha helical secondary structure and possessed a remarkable thermal stability and refolding capacity, a property that was greatly reduced after calcium depletion. Circular dichroism analysis of the calcium-bound and apo form of rAln g 4 indicated that calcium-induced modulation of **IgE binding** could be due to changes in the protein conformation. Purified rAln g 4 elicited dose-dependent basophil histamine release and immediate type skin reactions in sensitized patients. It may hence be useful for allergy diagnosis and for specific immunotherapy.

L30 ANSWER 38 OF 77 MEDLINE

1999057827 Document Number: 99057827. PubMed ID: 9837853. Peptide mimotopes displayed by phage inhibit antibody **binding** to bet v 1, the major birch pollen **allergen**, and induce specific IgG response in mice. Jensen-jarolim E; Leitner A; Kalchhauser H; Zurcher A; Ganglberger E; Bohle B; Scheiner O; Boltz-nitulescu G; Breiteneder H. (Department of General and Experimental Pathology, University of Vienna, Austria.. erika.jensen-jarolim@akh.wien.ac.at) . FASEB JOURNAL, (1998 Dec) 12 (15) 1635-42. Journal code: 8804484. ISSN: 0892-6638. Pub. country: United States. Language: English.

AB The major birch pollen **allergen** Bet v 1 is one of the most

extensively characterized **allergens** both on the molecular and the immunological level. To define conformational **B cell epitopes** on Bet v 1, we screened filamentous phage libraries expressing circular or linear nonapeptides to select ligands specific for anti-Bet v 1 murine monoclonal antibodies BIP1 and BIP4. The deduced amino acid sequence of the BIP1 ligand was CFPYCYPSESA, and of the BIP4-ligand, CRQTRTMPGC. Both sequences derived from the circular phage library. Alignments to the sequence of Bet v 1 showed no similarities, indicating that the antibodies most likely recognize discontinuous epitopes. Phages displaying these mimotopes were capable of inhibiting interactions of the anti-Bet v 1 monoclonals with Bet v 1 in a dose-dependent manner in ELISA. In contrast, sequence-identical synthetic peptides were ineffective in blocking the antibody-**allergen** interactions. This is in agreement with the conformational inhomogeneity of the peptides in solution as observed by nuclear magnetic resonance spectroscopy. Intragastric administration of phages expressing the BIP1 mimotope induced a Bet v 1-specific IgG response in Balb/c mice. We conclude that peptide mimotopes, when displayed on phages, may induce a protective IgG response preventing **IgE**-mediated allergic reactions, suggesting a possible clinical application.

L30 ANSWER 39 OF 77 SCISEARCH COPYRIGHT 2002 ISI (R)

1998:934236 The Genuine Article (R) Number: 144TL. Immunodominant peptide epitopes of **allergen**, Asp f 1 from the fungus Aspergillus fumigatus. Kurup V P (Reprint); Banerjee B; Murali P S; Greenberger P A; Krishnan M; Hari V; Fink J N. VET ADM MED CTR, RES SERV 151I, 5000 W NATL AVE, MILWAUKEE, WI 53295 (Reprint); MED COLL WISCONSIN, DEPT MED, DIV ALLERGY IMMUNOL, MILWAUKEE, WI 53226; DEPT VET AFFAIRS MED CTR, MILWAUKEE, WI; NORTHWESTERN UNIV, SCH MED, DIV ALLERGY IMMUNOL, CHICAGO, IL; WAYNE STATE UNIV, DEPT BIOL SCI, DETROIT, MI 48202. PEPTIDES (DEC 1998) Vol. 19, No. 9, pp. 1469-1477. Publisher: ELSEVIER SCIENCE INC. 655 AVENUE OF THE AMERICAS, NEW YORK, NY 10010. ISSN: 0196-9781. Pub. country: USA. Language: English.

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Aspergillus fumigatus ribotoxin Asp f 1 is a major **allergen** with **IgE binding** activity to serum of a majority of patients with allergic bronchopulmonary aspergillosis (ABPA). The **IgE binding** epitopes or the T-cell stimulatory peptides of this molecule have not been studied. In the present investigation, we have synthesized linear decapeptides spanning the whole molecule of Asp f 1 and analyzed their **IgE binding** properties. We have also synthesized peptides based on their possible T-cell stimulatory properties and studied the stimulation of peripheral blood mononuclear cells from ABPA patients and normal controls. Several peptides demonstrated distinct **IgE** antibody **binding** response against sera from ABPA patients and proliferative response against peripheral blood mononuclear cells from the patients. From the results, it can be concluded that the carboxy-terminal region of Asp f 1 representing amino acid residues 115-149 involved in both humoral and cell mediated immunoresponses in ABPA. (C) 1998 Elsevier Science Inc.

L30 ANSWER 40 OF 77 CAPLUS COPYRIGHT 2002 ACS

1998:729809 Document No. 130:137849 Parietaria pollen **allergens**. Colombo, P.; Duro, G.; Costa, M. A.; Izzo, V.; Mirisola, M.; Locorotondo, G.; Cocchiara, R.; Geraci, D. (Istituto di Biologia dello Sviluppo, CNR, Palermo, 90146, Italy). Allergy (Copenhagen), 53(10), 917-921 (English) 1998. CODEN: LLRGDY. ISSN: 0105-4538. Publisher: Munksgaard International Publishers Ltd..

AB A review with 31 refs. Discussed are: Parietaria officinalis (Po) and P. judaica (Pj) native major **allergens**; cDNA cloning; immunol. characterization of the recombinant **allergens**; **B cell epitope** and 3-D structure; and T cell epitope. The isolation and characterization of **allergens** have offered the

following aids to immunotherapeutic research: (1) the means to produce large amts. of highly purified **allergens** for desensitization studies; (2) the means to isolate **B cell epitope** capable of **binding** selectively either IgG or **IgE**; and (3) the elucidation of T cell-specific recognition and the design of modified **allergens** capable of activating Th1 responses.

L30 ANSWER 41 OF 77 MEDLINE

1998202668 Document Number: 98202668. PubMed ID: 9541587. Differential regulation of human T cell cytokine patterns and **IgE** and IgG4 responses by conformational antigen variants. Akdis C A; Blesken T; Wymann D; Akdis M; Blaser K. (Swiss Institute of Allergy and Asthma Research, Davos, Switzerland.. akdisac@siaf.unizh.ch) . EUROPEAN JOURNAL OF IMMUNOLOGY, (1998 Mar) 28 (3) 914-25. Journal code: 1273201. ISSN: 0014-2980. Pub. country: GERMANY: Germany, Federal Republic of. Language: English.

AB Bee venom phospholipase A2 (PLA) represents the major **allergen** and antigen in allergic and non-allergic individuals sensitized to bee sting. We have studied specific activation of peripheral T cells by different structural and conformational variants of PLA and secretion of cytokines regulating **IgE** and IgG4 antibody (Ab) formation. PLA molecules expressing the correctly folded tertiary structure, which show high affinity to membrane phospholipids and were recognized by Ab from bee sting allergic patients, induced high IL-4, IL-5 and IL-13 production in peripheral blood mononuclear cell cultures. In contrast, non-refolded recombinant PLA (rPLA) and reduced and alkylated native PLA (nPLA) induced more IFN-gamma and IL-2 and higher proliferative responses. Differences in proliferation and cytokine patterns among correctly folded and non-refolded PLA resulted from conformation-dependent involvement of different antigen-presenting cell (APC) types. Antigen (Ag)-presenting B cells recognized PLA only in its natural conformation, stimulated Th2 type cytokines and induced **IgE** Ab. Non-refolded PLA was recognized, processed and presented exclusively by monocytes and induced a Th1 dominant cytokine profile leading to IgG4 production by B cells. The possibility that production of particular cytokine patterns and Ig isotype was influenced by the enzymatic activity of PLA was excluded by using enzymatically inactive H34Q point-mutated, refolded rPLA. These findings demonstrate the decisive role of specific Ag recognition by different APC, depending on structural features, membrane phospholipid **binding** property and the existence of conformational **B cell epitopes**, in the differential regulation of memory **IgE** and IgG4 Ab. Furthermore, they show that a change from **IgE** -mediated allergy to normal immunity against a major **allergen** can be induced by rPLA variants that are not recognized by specific Ab and B cells but still carry the T cell epitopes. These features may enable new applications for safer immunotherapy.

L30 ANSWER 42 OF 77 MEDLINE

1999068569 Document Number: 99068569. PubMed ID: 9853680. A bovine dander **allergen**, comparative modeling, and similarities and differences in folding with related proteins. Santa H; Saarela J T; Laatikainen R; Rautianen J; Virtanen T; Rytkonen M; Mantyjarvi R. (Department of Chemistry, University of Kuopio, Finland.. santa@kummeli.uku.fi) . JOURNAL OF PROTEIN CHEMISTRY, (1998 Oct) 17 (7) 657-62. Journal code: 8217321. ISSN: 0277-8033. Pub. country: United States. Language: English.

AB The most important allergenic protein in cow dander and urine is Bos d 2. It is proposed to belong to the family of lipocalins, which are proteins capable of **binding** small hydrophobic molecules. The allergenic properties of Bos d 2 indicate an interaction between the accessible regions of the native protein and **IgE**. In this work, a three-dimensional model was created for Bos d 2 by comparative modeling, and features characteristic of outlier lipocalins were observed. The

protruding regions of the surface were characterized and used in predicting the possible **B-cell epitopes**. There is a pocket inside the core and its size is appropriate for small molecules. The model shows a hydrophilic amino acid side chain of glutamic acid 115 on the inner surface of the hole and a phenylalanine as the "gatekeeper" instead of tyrosine, which is common in experimentally modeled lipocalins.

L30 ANSWER 43 OF 77 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.
1998269541 EMBASE Molecular biology of Aspergillus **allergens**.
Banerjee B.; Kurup V.P.. Dr. B. Banerjee, Research Service 151-I, Department of Veterans Affairs, Medical Center, 5000 West National Avenue, Milwaukee, WI 53295, United States. banerjee@post.its.mcw.edu. Immunology and Allergy Clinics of North America 18/3 (601-618) 1998.
Refs: 99.
ISSN: 0889-8561. CODEN: INCAEP. Pub. Country: United States. Language: English. Summary Language: English.
AB The unavailability of standardized and reproducible allergenic extracts is the major limitation in the immunodiagnosis of ABPA. Through molecular cloning and expression of several A. fumigatus **allergens**, it may be possible to obtain well defined A. fumigatus **allergens** in large quantities for use in diagnosis. Two recombinant A. fumigatus **allergens**, Asp f 1 and Asp f 2, showed specific **IgE binding** in patients with ABPA comparable with their native counterparts. Over 12 recombinant A. fumigatus **allergens** have been identified and characterized; these can be used as a standardized allergenic preparation. Knowledge of the primary structure of these **allergens** facilitates the identification of immunodominant T- and **B-cell epitopes**, and may be used to unravel the structure-function relationship of these **allergens** in relation to immune modulation and intervention of the disease.

L30 ANSWER 44 OF 77 CAPLUS COPYRIGHT 2002 ACS
1998:141656 Characterization and epitope analysis of ARA h 3, a glycinin involved in peanut hypersensitivity.. Helm, Erica M.; Rabjohn, Pat A.; Stanley, J. Steven; West, C. Michael; Huang, S. K.; Sampson, H.; Burks, A. Wesley; Bannon, Gary A. (Department Chemistry, Hendrix College, Conway, AR, 72032, USA). Book of Abstracts, 215th ACS National Meeting, Dallas, March 29-April 2, CHED-179. American Chemical Society: Washington, D. C. (English) 1998. CODEN: 65QTAA.
AB Peanut allergy is a major health concern due to the severity of the allergic reaction, the lifelong persistence of the allergy, and the ubiquitous use of peanut as a protein supplement in processed foods. Using a previously unidentified peanut **allergen**, Ara h 3 cDNA clone was isolated, sequenced and found to be 1530 nucleotides and encoded a 510 amino acid protein. This sequence showed homol. to the glycinin family of seed storage proteins of common legumes. Synthetic peptides were used to det. which regions of the primary sequence served as linear **B-cell epitopes** for **binding** serum **IgE** from a population of peanut hypersensitivity patients. These epitopes were distributed evenly throughout the primary sequence and were six to ten amino acids in length. Further studies will be focused on identifying individual amino acids crit. for **IgE binding**. Once these amino acids are identified, it will be possible to mutate crit. residues to eliminate the ability of this protein to bind **IgE**.

L30 ANSWER 45 OF 77 MEDLINE
1999051194 Document Number: 99051194. PubMed ID: 9831803. **IgE binding** capacity of synthetic and recombinant peptides of the major storage mite (Lepidoglyphus destructor) **allergen**, Lep d 2.
Elfman L H; Whitley P; Schmidt M; van Hage-Hamsten M. (Department of Laboratory Medicine, Division of Clinical Immunology, Karolinska Hospital

and Institute, Stockholm, Sweden.) INTERNATIONAL ARCHIVES OF ALLERGY AND IMMUNOLOGY, (1998 Nov) 117 (3) 167-73. Journal code: 9211652. ISSN: 1018-2438. Pub. country: Switzerland. Language: English.

AB BACKGROUND: Lepidoglyphus destructor is an important non-pyroglyphid mite species in Europe and a dominant **allergen** in farming environments. The major **allergen** of L. destructor, Lep d 2, is a protein of 13.2 kD that is recognised by about 90% of sera RAST positive to this mite species. METHODS: The cDNA of two isoallergens of the Lep d 2 has previously been sequenced and the protein expressed in different protein expression systems. In order to map the **B-cell epitopes**, the full length protein and the truncated forms of the protein have been expressed in Escherichia coli as glutathione-S-transferase (GST) fusion proteins. Recombinant Lep d 2 fragments and synthetic overlapping 15 mer peptides spanning Lep d 2 were probed with sera from patients allergic to storage mite. RESULTS: The full-length (125 amino acids) GST fusion protein reacted strongly with patient **IgE** in Western blots and dot blots. Synthetic peptides failed to react with **IgE** antibodies from mite-allergic patients and the truncated fusion proteins displayed weak **IgE-binding** capacity. CONCLUSION: We conclude that there are no dominant linear **IgE-binding** epitopes in Lep d 2. Recombinant or synthetic Lep d 2 fragments may, however, be further evaluated as hypoallergenic candidate molecules for specific immunotherapy.

L30 ANSWER 46 OF 77 MEDLINE

1999041370 Document Number: 99041370. PubMed ID: 9825997. B- and T-cell epitopes of tropomyosin, the major shrimp **allergen**. Subba Rao P V; Rajagopal D; Ganesh K A. (Department of Biochemistry, Indian Institute of Science, Vittal Mallya Scientific Research Foundation, Bangalore.) ALLERGY, (1998) 53 (46 Suppl) 44-7. Ref: 20. Journal code: 7804028. ISSN: 0105-4538. Pub. country: Denmark. Language: English.

AB The major crustacean **allergen** characterized from different species of shrimp is the muscle protein tropomyosin. Two shared epitopes corresponding to 47-63 and 150-158 of the deduced amino-acid sequence of the brown shrimp, M. ensis, were identified as **IgE-binding B-cell epitopes**. A 21-mer peptide spanning the amino-acid residues 261-281 was identified as a putative T-cell epitope capable of reducing ongoing tropomyosin-specific IgG and **IgE** responses in a mouse model. These observations suggest that peptide immunotherapy may also be effective in the treatment of food hypersensitivity.

L30 ANSWER 47 OF 77 MEDLINE

1998426313 Document Number: 98426313. PubMed ID: 9751845. Cellular and molecular characterization of a major soybean **allergen**. Helm R; Cockrell G; Herman E; Burks A; Sampson H; Bannon G. (Department of Pediatrics, University of Arkansas for Medical Sciences, Arkansas Children's Hospital Research Institute, Little Rock, Ark., USA.) INTERNATIONAL ARCHIVES OF ALLERGY AND IMMUNOLOGY, (1998 Sep) 117 (1) 29-37. Journal code: 9211652. ISSN: 1018-2438. Pub. country: Switzerland. Language: English.

AB Soybean proteins share a large number of cross-reacting **allergens** with other members of the legume family; however, soy-allergic patients rarely react clinically to other members of the legume family. Gly m Bd 30K, an **IgE-binding** protein with a molecular weight of 30 kD, was identified in soybean extracts by Western **IgE** -immunoblot analysis. This monomeric **allergen** was shown to have an N-terminal amino acid sequence and amino acid composition identical to that of the seed 34-kD protein, P34, a thiol protease of the papain family. Electron-microscopic immunolocalization of P34 monoclonal antibodies and **IgE binding** to sections of soybean seeds showed dense staining throughout the vacuolar bodies, localizing the **allergens** in protein storage vacuoles of seed cotyledons. We used

pooled serum from soybean-sensitive patients to determine the linear **IgE**-specific epitopes in the 34-kD **allergen** amino acid sequence. **B-cell epitope** mapping revealed 10 regions of **IgE-binding** activity using an overlapping peptide strategy of 15-mers offset by 8 amino acids throughout the P34 sequence. Smaller overlapping peptides, 10-mers offset by 2 amino acids, revealed 16 distinct linear epitopes, 9 of which were mapped to the mature protein. No obvious amino acid sequence motifs could be identified by the smaller **IgE-binding** epitopes. Using individual patient serum, 5 immunodominant epitopes were identified in this **allergen**
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L30 ANSWER 48 OF 77 CAPLUS COPYRIGHT 2002 ACS
1998:530010 T and B lymphocyte epitopes of food **allergens**..
Raybourne, R. B. (Food and Drug Administration, Center Food Safety and Applied Nutrition, Laurel, MD, 20708, USA). Book of Abstracts, 216th ACS National Meeting, Boston, August 23-27, AGFD-025. American Chemical Society: Washington, D. C. (English) 1998. CODEN: 66KYA2.
AB An **IgE**-mediated allergic response is the result of interactions between T and B lymphocytes and antigen processing and presenting cells. The regions of a protein antigen, recognized by the T lymphocyte receptor (TCR) and the **IgE** antibody mol. are called epitopes. The epitopes recognized by T lymphocytes are usually, but not always, different from those recognized by B cells. **B cell epitopes** can be based on primary linear amino acid sequence (continuous), or on secondary structure produced by protein folding (conformational epitopes). CD4 T cells recognize their epitopes in the form of small peptides which are located in a peptide **binding** groove, formed by folding of Major Histocompatibility Class II mols. These peptides are produced by degrdn. of the whole protein antigen within antigen processing cells Most **allergens** studies thus far contain multiple, different T cell epitopes. The T cell responses that govern whether or not an allergic response will result from T and B cell interaction are not fully understood.

L30 ANSWER 49 OF 77 MEDLINE
1998426310 Document Number: 98426310. PubMed ID: 9751842. Determinants and mechanisms of human immune responses to bee venom phospholipase A2. Blaser K; Carballido J; Faith A; Crameri R; Akdis C. (Swiss Institute of Allergy and Asthma Research (SIAF), Davos, Switzerland.. kblaser@siaf.unizh.ch) . INTERNATIONAL ARCHIVES OF ALLERGY AND IMMUNOLOGY, (1998 Sep) 117 (1) 1-10. Ref: 43. Journal code: 9211652. ISSN: 1018-2438. Pub. country: Switzerland. Language: English.
AB The elicitation of an immune response to protein antigens depends on the specific recognition of antigenic determinants (epitopes) by T and B lymphocytes. Bee venom phospholipase A2 (PLA) represents the major antigen/**allergen** of honey bee venom. It displays three dominant immunogenic peptide and one glycopeptide T cell recognition sites. These epitopes are equally recognized by both allergic and nonallergic individuals. A mixture of the three epitope containing peptides was successfully used in specific immunotherapy of bee venom-allergic patients. Both peptide and whole bee venom immunotherapy induced a state of specific anergy in T cells. The production of specific **IgE** and IgG4 antibodies directly correlated with the secreted interleukin-4:gamma-interferon (IL-4:IFNgamma) ratio, which itself depended on the concentration of available antigen and the strength of the T cell-activating signal. This signal comprises accumulated molecular interactions delivered by engagement of the antigenic peptide/MHC class II complex with the T cell receptor (TcR). Indeed the thermodynamic laws of chemical equilibrium reactions reveal that the antigen concentration, together with the equilibration constant Ki and the related Gibbs standard free energy DeltaG degrees of the MHC-II/Ag/TcR complex reaction, may govern the secreted IL-4:IFNgamma ratio, and in consequence, differential

IgE and IgG4 antibody formation. Ki includes epitope and MHC-II haplotype variability and therefore represents a measure of immunological individuality. A major **B cell epitope** was determined by using point-mutated PLA. Specific antigen recognition by B cells can trigger distinct cytokine profiles in T cells and contribute to the differential regulation of specific **IgE** and IgG4 antibodies. Our results indicate that distinct cytokine profiles inducing allergic and nonallergic responses can be attributed to thresholds of T cell activation generated by the specific **binding** properties of individual MHC-II molecules to immunogenic T cell epitopes and their presentation to TcR.

L30 ANSWER 50 OF 77 CAPLUS COPYRIGHT 2002 ACS
1997:473729 Document No. 127:94502 Cloning, nucleotide and amino acid sequences, and immunoassays of peanut **allergens** causing hypersensitivity. Burks, A. Wesley, Jr.; Helm, Ricki M.; Cockrell, Gael; Stanley, J. Steven; Bannon, Gary A. (University of Arkansas, USA; Burks, A. Wesley, Jr.; Helm, Ricki M.; Cockrell, Gael; Stanley, J. Steven; Bannon, Gary A.). PCT Int. Appl. WO 9724139 A1 19970710, 352 pp. DESIGNATED STATES: W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1996-US15222 19960923. PRIORITY: US 1995-9455 19951229; US 1996-610424 19960304.

AB Crude Florunner exts. were fractioned by anion-exchange chromatog. using a step gradient. A protein peak which eluted at 10% NaCl and demonstrated intense **IgE-binding** was further analyzed by 2-dimensional SDS-PAGE/immunoblot anal. The majority of this fraction is a protein which has a mol. wt. of 17 kDa and a pI of 5.2. Sequencing data from the N-terminus revealed the following initial 9 amino acids: (*)-Q-Q-(*)-E-L-Q-D-L. Based on **IgE-binding** activity and no known amino acid sequence identity to other **allergens**, this **allergen** is designated Ara h II. Ara h II may be used to detect and quantify peanut **allergens** in foodstuffs. Serum **IgE** from patients with documented peanut hypersensitivity reactions and a peanut cDNA expression library were used to identify clones that encode peanut **allergens**. One of the major peanut **allergens**, Ara h I, was selected from these clones using Ara h I-specific oligonucleotides and PCR technol. The cDNA and deduced amino acid sequences are presented for Ara h I (a vicilin-like protein) and Ara h II (a conglutin-like protein). **B-cell epitope** mapping and monoclonal antibody prodn. allowed the development of efficient immunoassays, and the **allergens** can be used for vaccination therapy to treat peanut hypersensitivity in human patients.

L30 ANSWER 51 OF 77 MEDLINE
1998208297 Document Number: 98208297. PubMed ID: 9548517. **IgE** from latex-allergic patients binds to cloned and expressed **B cell epitopes** of prohevein. Banerjee B; Wang X; Kelly K J; Fink J N; Sussman G L; Kurup V P. (Allergy-Immunology Division of the Medical College of Wisconsin, Milwaukee 53226, USA.) JOURNAL OF IMMUNOLOGY, (1997 Dec 1) 159 (11) 5724-32. Journal code: 2985117R. ISSN: 0022-1767. Pub. country: United States. Language: English.

AB Prohevein is one of the major **allergens** associated with latex allergy. In the present study, we identified **IgE binding** regions of prohevein, and expressed multiple **IgE binding** epitopes by selective cloning. These truncated polypeptides were then used to demonstrate **IgE** in the sera of patients. Decapeptides of prohevein were synthesized on derivatized cellulose membrane with an

offset of one amino acid. The **IgE** reactivity of these linear peptides was evaluated separately using pooled sera from latex-allergic health care workers (HCW) and spina bifida (SB) patients. A total of 10 **IgE binding** epitopes representing unique as well as shared epitopes from both the N- and C-domains of the prohevein were identified. Recombinant polypeptides were constructed based on the identified epitopes, and clones carrying DNA fragments were overexpressed. These recombinant peptides were evaluated for **IgE binding** with sera from HCW, SB, and normal individuals. Recombinant prohevein, hevein, and the C-domain exhibited **IgE binding** in 84, 88, and 40% of HCW sera, respectively, as against reactivity of 84% with crude latex **allergens**. However, only 48% of the sera from SB patients showed **IgE binding** with recombinant prohevein, while 56 and 28% had reactivity with recombinant N- and C-domains, respectively. Among the three remaining recombinant peptides of the C-domain, only CA44-103 showed **IgE binding** with SB patients. The results of the present study suggest that linear **IgE** epitope analysis and construction of recombinant peptides increase the sensitivity and specificity of the immunodiagnosis of latex allergy and provide more information on the immunopathogenesis of hypersensitivity reaction mediated by type I allergy.

L30 ANSWER 52 OF 77 MEDLINE

97400376 Document Number: 97400376. PubMed ID: 9257870. Allergenic properties of ovomucoid in man. Cooke S K; Sampson H A. (Department of Pediatrics, Johns Hopkins University School of Medicine, Baltimore, MD 21287, USA.) JOURNAL OF IMMUNOLOGY, (1997 Aug 15) 159 (4) 2026-32. Journal code: 2985117R. ISSN: 0022-1767. Pub. country: United States. Language: English.

AB Ovomucoid, the dominant **allergen** in hen's egg, is a highly glycosylated protein comprising 186 amino acids arranged in three tandem domains (Gal d 1.1, 1.2, and 1.3). The purpose of this study was to evaluate the allergenic properties of ovomucoid. The three ovomucoid domains were isolated and evaluated with sera from egg allergic patients to determine B cell domain specificity, **B cell epitopes**, and the relative importance of linear and conformational structures and carbohydrate chains to **B cell epitopes**. Peripheral blood T cells from egg allergic patients were used to evaluate T-dominant domains and reactivity to reduced and oxidized ovomucoid. There was significantly more **IgE** activity to the second ovomucoid domain (median percentage of ovomucoid-specific **IgE**: Gal d 1.2, 40%; Gal d 1.1, 23%; Gal d 1.3, 26%). Quantities of patient IgG Ab were comparable for all three domains. Five **IgE** and seven IgG **binding** regions were identified. **IgE** Ab **binding** to reduced ovomucoid and IgG **binding** to oxidized ovomucoid were significantly reduced compared with that to native ovomucoid (28 and 69%, respectively). Peripheral blood T cells of 21 of 33 patients reacted to Gal d 1.3, 18 of 33 reacted to Gal d 1.2, and 18 of 33 reacted to Gal d 1.1. T cell proliferation in vitro in response to reduced and oxidized ovomucoid were significantly greater than that in response to the native protein. These results indicate a dichotomy between T and B cell domain dominance, and the presence of both unique and common **IgE** and IgG epitopes. Furthermore, the results suggest that conformational **B cell epitopes** play a more significant role in ovomucoid allergenicity than previously appreciated, and that carbohydrate moieties have a minor effect on allergenicity.

L30 ANSWER 53 OF 77 CAPLUS COPYRIGHT 2002 ACS

1997:347862 Document No. 127:80099 Extraordinary stability of **IgE-binding** Parietaria pollen **allergens** in relation to chemically bound flavonoids. Romano, M. L. Gonzalez; Gallego, M. T.; Berrens, L. (LETI, Res. Lab. C.B.F., Madrid, Spain). Molecular Immunology, Volume Date 1996, 33(17/18), 1287-1293 (English) 1997. CODEN:

MOIMD5. ISSN: 0161-5890. Publisher: Elsevier.

AB It is known that the skin-active and **IgE-binding** components in Parietaria pollen exts. are not restricted to the predominant protein **allergens** of Mr 12,000-15,000, but are present as well among the naturally occurring constituents of Mr <10,000. Indeed, the **IgE-binding** Parietaria pollen components are quite heterogeneous, ranging from high- to low-mol. mass, whereby the **IgE-binding** epitopes display an unusual chem. stability. Furthermore, the pollen of Parietaria species demonstrably contains a high proportion of flavonoid pigments. Since these pollen grains cannot be collected entirely free from non-pollen plant parts, the usual allergenic exts. of Parietaria encompass both the polyphenolic substrate mols. and the enzyme polyphenoloxidase as ingredients for the oxidative generation of flavonol-protein conjugates during the extn. process. Here, this is illustrated by spectroscopic analyses of the free and bound flavonoids in Parietaria pollen exts., as well as of the peptide fragments produced from the allergenic proteins by enzymic or chem. hydrolysis. None of these relatively harsh treatments had an effect on the **IgE-binding** properties of the allergenic (sub-)components, even though detectable proteins in isoelec. focusing and immunoblotting were lost. It is proposed that the extraordinary stability of **IgE-binding** Parietaria components over a wide mol. range may be attributed to chromophoric flavonoid side-chains as (part of) the corresponding **B-cell epitopes**.

L30 ANSWER 54 OF 77 MEDLINE

97358126 Document Number: 97358126. PubMed ID: 9215246. Mapping of **IgE-binding** epitopes on the recombinant major group I **allergen** of velvet grass pollen, rHol l 1. Schramm G; Bufe A; Petersen A; Haas H; Schlaak M; Becker W M. (Forschungszentrum Borstel, Germany.) JOURNAL OF ALLERGY AND CLINICAL IMMUNOLOGY, (1997 Jun) 99 (6 Pt 1) 781-7. Journal code: 1275002. ISSN: 0091-6749. Pub. country: United States. Language: English.

AB BACKGROUND: New and more successful approaches to diagnosis and therapy of allergic diseases require a more subtle understanding of the structure and the epitopes on the **allergen** molecule. OBJECTIVE: This study was done to obtain more information on the structure and the **IgE-binding** epitopes of a major **allergen** of velvet grass pollen, Hol l 1. METHODS: We cloned Hol l 1 from a complementary DNA library and performed **B-cell epitope** mapping with 21 recombinant fragments expressed as fusion proteins in Escherichia coli. The fragments were analyzed by Western blotting with sera from 50 different patients. RESULTS: The patients' sera individually recognized at least four different **IgE-binding** regions (amino acids 1 to 27, 61 to 76, 84 to 105, and 158 to 240). According to their **binding** patterns with these epitopes, they were divided into five groups. Most sera (92%) bound to the C-terminal peptide (158 to 240), which consists of more than 80 amino acids, whereas there was virtually no **binding** to smaller fragments covering this region. In contrast to the C-terminal peptide, the **IgE-binding** peptides on the N terminus and on the middle region of the molecule were of a smaller size (15 to 30 amino acids). CONCLUSIONS: The major group I **allergen** of velvet grass bears at least four different **IgE-binding** epitopes, which were individually recognized by sera from different patients. The C terminus represents the major **IgE-binding** region and contains at least one discontinuous **IgE-binding** epitope, whereas the N terminus and middle region of Hol l 1 seem to contain continuous **IgE-binding** epitopes.

L30 ANSWER 55 OF 77 MEDLINE

97368200 Document Number: 97368200. PubMed ID: 9224967. Determination of the N- and C-terminal sequences required to bind human **IgE** of

the major house dust mite **allergen** Der f 2 and epitope mapping for monoclonal antibodies. Takai T; Yuuki T; Okumura Y; Mori A; Okudaira H. (Bioscience Research and Development Laboratory, Biotechnology Section, Asahi Breweries Ltd, Ohta-ku, Tokyo, Japan.) MOLECULAR IMMUNOLOGY, (1997 Feb) 34 (3) 255-61. Journal code: 7905289. ISSN: 0161-5890. Pub. country: ENGLAND: United Kingdom. Language: English.

AB **B cell epitopes** of the major house dust mite **allergen** Der f 2 from Dermatophagoides farinae were analysed using deletion mutants of Der f 2 expressed as fusion proteins in Escherichia coli. The reactivities of these partial Der f 2 molecules to human anti-mite **IgE** antibodies in atopic patients and to murine anti-Der f2 monoclonal antibodies (mAbs) were examined by immunoblotting. A C-terminal deletion mutant of Der f 2, 1-123, had almost the same reactivity to human **IgE** as the whole Der f 2 (1-129) and an N-terminal deletion mutant of Der f 2 (25-129) still had weak reactivity. On the other hand, in two deleted Der f 2 molecules, 1-120 and 30-129, reactivity was lost in spite of long overlapping sequences. These results suggest that the human **IgE** antibodies to Der f 2 in atopic patient sera recognize the conformational structures dependent on the tertiary structure of Der f 2, including disulfide bond formations, rather than the contiguous sequences of amino acids. The sequences 1-24, 25-29 and 121-123 were revealed as the minimum N- and C- terminal amino acid sequences required for **IgE binding**. Contrastingly, all three murine mAbs bound to the smaller deletion mutants, 1-90 and 67-129, suggesting that the cores of the epitopes for these mAbs exist in the 24 amino acid sequence of Der f 2, 67-90 overlapping the sequential human **IgE** epitope on Der p 2, the equivalent **allergen** from Dermatophagoides pteronyssinus. These findings are important for the understanding of the antigenic structure of Der f 2 and for the manipulation of the **allergen** for immunotherapy.

L30 ANSWER 56 OF 77 CAPLUS COPYRIGHT 2002 ACS

1998:145080 Document No. 128:242662 Therapy of type I allergy by interfering with the immunoglobulin E-**allergen** interaction. Valenta, Rudolf; Wiedemann, Petra; Ball, Tanja; Laffer, Sylvia; Dolecek, Christiane; Steinberger, Peter; Flicker, Sabine; Vrtala, Susanne; Spitzauer, Susanne; Kraft, Dietrich (Institute of General and Experimental Pathology, University of Vienna, Vienna, Austria). Progress in Allergy and Clinical Immunology, Proceedings of the International Congress of Allergology and Clinical Immunology, 16th, Cancun, Mex., Oct. 19-24, 1997, 144-149. Editor(s): Oehling, Albert K.; Huerta Lopez, J. G. Hogrefe & Huber: Seattle, Wash. (English) 1997. CODEN: 65SQAB.

AB A review with 41 refs. The **IgE-allergen** interaction represents the crucial event that triggers mediator release from allergic effector cells, and thus causes immediate-type reactions. Recent advances in the field of mol. **allergen** characterization indicate that due to extensive cross-reactivities among **allergens**, a limited panel of **B-cell epitopes** may be defined for diagnosis and probably therapy of allergy. An increasing no. of recombinant **allergens** is currently being produced, and progress has been made in understanding **allergen**-antibody interactions. Current approaches to specific immunotherapy are mainly dedicated to the T-cell mediated control of **IgE**-prodn. by either shifting the increased Th2 activity in allergic patients to a Th1 state or by induction of T-cell tolerance or anergy. The authors propose that the **IgE -allergen** interaction may serve as another possible target for specific therapeutic intervention. Interfering with the **IgE-allergen** interaction may be a promising strategy, because **IgE** represents the least abundant class of Igs, and allergic reactions mostly take place on mucosal surfaces in organs, which are easily accessible. Non-anaphylactic **allergen**-fragments that contain **binding** sites for **IgE** antibodies of only one specificity may be used to sat. effector cells prior to **allergen**

contact or to induce blocking IgG-antibodies by active immunization. Blocking antibodies or antibody fragments obtained from human or animal systems by tissue culture techniques or by combinatorial library technol. may be used for passive therapy in **allergen**-exposed tissues.

L30 ANSWER 57 OF 77 MEDLINE
97315883 Document Number: 97315883. PubMed ID: 9171888. Extraordinary stability of **IgE-binding** Parietaria pollen **allergens** in relation to chemically bound flavonoids. Gonzalez Romano M L; Gallego M T; Berrens L. (Research Laboratories C.B.F. LETI, Tres Cantos, Madrid, Spain.) MOLECULAR IMMUNOLOGY, (1996 Dec) 33 (17-18) 1287-93. Journal code: 7905289. ISSN: 0161-5890. Pub. country: ENGLAND: United Kingdom. Language: English.

AB It is known that the skin-active and **IgE-binding** components in Parietaria pollen extracts are not restricted to the predominant protein **allergens** of M(r) 12000-15000, but are present as well among the naturally occurring constituents of M(r) < 10000. Indeed, the **IgE-binding** Parietaria pollen components are quite heterogeneous, ranging from high- to low-molecular mass, whereby the **IgE-binding** epitopes display an unusual chemical stability. Furthermore, the pollen of Parietaria species demonstrably contain a high proportion of flavonoid pigments. Since these pollen grains cannot be collected entirely free from non-pollen plant parts, the usual allergenic extracts of Parietaria encompass both the polyphenolic substrate molecules and the enzyme polyphenoloxidase as ingredients for the oxidative generation of flavonol-protein conjugates during the extraction process. In the present work this is illustrated by spectroscopic analyses of the free and bound flavonoids in Parietaria pollen extracts, as well as of the peptide fragments produced from the allergenic proteins by enzymatic or chemical hydrolysis. None of these relatively harsh treatments had a significant effect on the **IgE-binding** properties of the allergenic (sub-)components, even though detectable proteins in isoelectric focusing and immunoblotting were lost. It is proposed that the extraordinary stability of **IgE-binding** Parietaria components over a wide molecular range may be attributed to chromophoric flavonoid side-chains as (parts of) the corresponding **B-cell epitopes**.

L30 ANSWER 58 OF 77 SCISEARCH COPYRIGHT 2002 ISI (R)
96:623612 The Genuine Article (R) Number: VC478. MOLECULAR-BIOLOGY OF **ALLERGENS**. BUSH R K (Reprint). WILLIAM S MIDDLETON MEM VET ADM MED CTR, DEPT ALLERGY, 2500 OVERLOOK TERRACE, MADISON, WI, 53705 (Reprint); UNIV WISCONSIN, DEPT MED, MADISON, WI, 00000. IMMUNOLOGY AND ALLERGY CLINICS OF NORTH AMERICA (AUG 1996) Vol. 16, No. 3, pp. 535. ISSN: 0889-8561. Pub. country: USA. Language: ENGLISH.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Molecular cloning has resulted in an ever-increasing number of purified **allergens**. Their functional and biologic characteristics can be determined and cross-reactivity predicted. Recombinant **allergens** can lead to improved diagnostic reagents. Studies of allergenic T- and **B-cell epitopes** may produce new therapeutic approaches to allergic diseases.

L30 ANSWER 59 OF 77 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
1996:261971 Document No.: PREV199698818100. Allergenic and antigenic determinants of latex **allergen** Hev b 1: Peptide mapping of epitopes recognized by human, murine and rabbit antibodies. Chen, Z. (1); Van Kampen, V.; Raulf-Heimsoth, M.; Baur, X.. (1) BGFA, Buerkle-de-la-Camp-Platz 1, 44789 Bochum Germany. Clinical and Experimental Allergy, (1996) Vol. 26, No. 4, pp. 406-415. ISSN: 0954-7894. Language: English.

AB Background: The rubber elongation factor in Hevea rubber (Hev b 1) is one of the most important latex **allergen** and is leading cause of

latex type 1 hypersensitivity in children with spina bifida. Objective: The aim of this study was to define the allergenic and antigenic epitopes of Hev b 1. Methods: The immunoglobulin- (Ig)E and IgG antibody **binding** sites on Hev b 1 **allergen** were delineated by enzyme linked immunosorbent assay (ELISA) using synthetic overlapping peptides covering the whole Hev b 1 sequence. In order to improve the **binding** capacity and specificity all peptides were biotinylated at the N-terminal end via a 6-aminohexanoic acid as spacer and then adsorbed to streptavidin pre-coated microtitre plates. Fine mapping to define the essential amino acid residues for the antibody **binding** was achieved by using overlapping peptides with one amino acid offset. Results: It was demonstrated that the **IgE** epitopes were located in different regions of Hev b 1 including the C-terminal segment (121-137) and the segments with amino acid residues of 30-49 and 46-64. Two monoclonal antibodies (MoAbs) II2F3 and II4G9 raised against purified Hev b 1 recognized the C-terminal segment only. The results of epitope mapping with three rabbit antisera revealed that five positive peptides, including the epitope peptides 31-49, 46-64 and 121-137, were involved in the antibody-**binding** sites. Fine mapping on the segments 46-64 and 121-137 showed that the two MoAbs reacted with the peptide 125-134 in the C-terminal region, whereas the peptide with amino acids 124-134 was essential for recognition by human **IgE** antibodies. Epitopes to rabbit polyclonal IgG and human **IgE** were also found to be involved in the amino acid residues of 47-59. Conclusion: Our results indicate that the most allergenic/antigenic portions of Hev b 1 **allergen** are the C-terminal region and the region with amino acid residues of 31-64. In both regions, the minimal **IgE**-**binding** epitope is almost identical with the IgG-**binding** epitope.

L30 ANSWER 60 OF 77 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
1997:26116 Document No.: PREV199799325319. Characterization of purified recombinant Bet v 1 with authentic N-terminus, cloned in fusion with maltose-**binding** protein. Spangfort, Michael D. (1); Ipsen, Henrik; Sparholt, Susanne H.; Aasmul-Olsen, Stig; Larsen, Martin R.; Mortz, Ejvind; Roepstorff, Peter; Larsen, Jorgen N.. (1) Res. Dep., ALK-ABELLO Group, Boge Alle 10-12, DK-2970 Horsholm Denmark. Protein Expression and Purification, (1996) Vol. 8, No. 3, pp. 365-373. ISSN: 1046-5928. Language: English.

AB A gene encoding the pollen major **allergen** Bet v 1 from Betula verrucosa (White Birch) has been cloned and expressed in Escherichia coli as a fusion with maltose-**binding** protein and a Factor Xa proteolytic cleavage site. A generally applicable cloning strategy based on polymerase chain reaction was designed to position the Factor Xa proteolytic site so that the authentic amino terminus of Bet v 1 was generated after cleavage. Fusion protein was isolated by amylose affinity chromatography and enzymatically cleaved by incubation with Factor Xa. Recombinant Bet v 1 was isolated by gel filtration and gave rise to a single band with apparent molecular weight of 17 kDa when analyzed by SDS-polyacrylamide gel electrophoresis. N-terminal sequencing of the first 20 amino acids showed complete agreement with the deduced Bet v 1 DNA sequence. Mass spectrometry showed that recombinant Bet v 1 has a molecular mass of 17,440 +- 2 Da; 86% of the recombinant Bet v 1 amino acid sequence could be verified by digestion with Lys-C and mass spectrometric peptide mapping. The yield of purified recombinant Bet v 1 was 10 mg per liter E. coli cell culture. Two-dimensional gel electrophoresis of purified recombinant protein gave rise to one major protein spot and one or two minor spots focusing at slightly different pHs. The immunochemical properties of recombinant protein were indistinguishable from those of naturally occurring Bet v 1 when compared using a panel of murine monoclonal antibodies and serum **IgE** from birch pollen allergic patients. Furthermore, recombinant Bet v 1 elicited T-cell proliferation comparable to that of natural Bet v 1. Thus, the

methods used for bacterial expression and protein purification result in relatively high yields of folded recombinant Bet v 1 with correct N-terminal sequence and molecular mass. Furthermore, the B- and T-cell epitope structures of recombinant Bet v 1 closely resemble those of the natural protein from pollen.

L30 ANSWER 61 OF 77 MEDLINE

96433347 Document Number: 96433347. PubMed ID: 8836334. Allergenic epitopes of bovine alpha S1-casein recognized by human **IgE** and IgG. Spuergin P; Mueller H; Walter M; Schiltz E; Forster J. (University Children's Hospital, Freiburg im Breisgau, Germany.) ALLERGY, (1996 May) 51 (5) 306-12. Journal code: 7804028. ISSN: 0105-4538. Pub. country: Denmark. Language: English.

AB **B-cell epitopes** of bovine alpha S1-casein, one of the major **allergens** of cow's milk, were identified by a screening method based on synthetic peptides. According to the known amino acid sequence of alpha S1-casein, a set of 188 overlapping sequential decapeptides shifted by one amino acid was manually synthesized on polyethylene pins by the 9-fluorenyl-methoxycarbonyl (Fmoc) method. Peptides were screened by an enzyme-linked immunosorbent assay (ELISA) specific for human **IgE** and IgG. Bound antibodies were detected by successive incubation with up to three polyclonal antibodies, the last one conjugated to horseradish peroxidase. Tested sera were from 15 patients with acute clinical reactions to cow's milk and **IgE** -specific reactions to bovine alpha-casein in the ELISA and immunoblot. Sera from 10 healthy subjects without remarkable reactions to cow's milk proteins were used as controls. All sera from allergic subjects showed reactions with three regions of alpha S1-casein, corresponding to amino acids 19-30, 93-98, and 141-150. Furthermore, individual sera showed reactions with other parts of the protein. No essential differences in the epitope specificity of **IgE** and IgG were found. Inhibition of **IgE binding** to alpha S1-casein with soluble synthetic peptides confirmed the results and revealed peptide CN-2 as the most inhibiting one.

L30 ANSWER 62 OF 77 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.

1996:149310 Document No.: PREV199698721445. Characterization of a dominant antigenic determinant of Par o I encoded by recombinant DNA. D'Abusco, A. Scotto; De Santo, C.; Menna, T.; Coscia, M. R.; Oreste, U.; Geller-Bernstein, C.; Ruffilli, A. (1). (1) IIGB, CNR, Via Marconi 10, Napoli 80125 Italy. Clinical and Experimental Allergy, (1996) Vol. 26, No. 2, pp. 223-231. ISSN: 0954-7894. Language: English.

AB Background: The pollens from Parietaria judaica and Parietaria officinalis are a major cause of pollinosis in Europe. Par o I (13.5 kDa) and Par j I (12 kDa), the major **allergens** from these species, are highly crossreactive. Methods: We have immunoscreened a P.judaica pollen cDNA expression library with a rabbit antiserum specific for Parj I and with a serum pool from allergic patients. An immunopositive clone containing a 26 bp insert was further characterized. The insert sequence was determined and the beta-galactosidase fusion protein was partially purified by electroelution from sodium dodecyl sulfate - polyacrylamide gel electrophoresis (SDS-PAGE) gels. Results: This fusion protein specifically and extensively inhibited Par o I and Par j I **binding** of a rabbit antiserum and of a serum pool obtained from allergic patients. The antifusion-protein antiserum obtained in a rabbit (anti 6a) specifically precipitated radioiodinated purified Par o I in the double antibody radioimmunoassay (DARIA) and competed with antibodies of sera from allergic patients for the **binding** to Parietaria pollen extract **allergens** by enzyme linked immunosorbent assay (ELISA). We investigated the prevalence of antibody response towards the 6a epitope in patients naturally sensitized to Parietaria. The presence of 6a specific **IgE** antibodies was assessed in the sera of 33 patients using inhibition assays. All sera had antibodies with this specificity: the

extensive percentage of inhibition reached suggested that they dominated individual ab response. Conclusion: In conclusion, the antibody response induced by natural exposure to the pollen of Parietaria appears to be highly focused on a single linear antigenic determinant of the major **allergens** which may play a relevant role in the development of clinical allergy. This report is, to our knowledge, the first description of a dominant linear epitope of a major **allergen**.

L30 ANSWER 63 OF 77 MEDLINE

95318399 Document Number: 95318399. PubMed ID: 7797791. **IgE** antibodies to recombinant forms of Fel d I: dichotomy between fluid-phase and solid-phase **binding** studies. Slunt J B; Rogers B L; Chapman M D. (Department of Medicine, University of Virginia, Charlottesville 22908, USA.) JOURNAL OF ALLERGY AND CLINICAL IMMUNOLOGY, (1995 Jun) 95 (6) 1221-8. Journal code: 1275002. ISSN: 0091-6749. Pub. country: United States. Language: English.

AB BACKGROUND: The major cat **allergen** Fel d I consists of two polypeptide chains linked by disulfide bonds, each of which has been expressed in bacteria. To investigate the antigenic structure of Fel d I, antibody **binding** to the native molecule and to each recombinant chain were compared. METHODS: Polyclonal human **IgE** and IgG antibodies and monoclonal antibodies (mAbs) to Fel d I were compared for **binding** to Fel d I, chain 1, or chain 2 by fluid-phase inhibition radioimmunoassay, RAST, and immunoabsorption. RESULTS: In the fluid-phase assay, neither recombinant chain significantly inhibited the **binding** of antibody to native Fel d I at concentrations of up to 10 micrograms/ml. Partial inhibition was observed when chain 1 was used, which inhibited the **binding** of two mAbs by 40% and 75%. In contrast, when the solid-phase RAST assay was used, **IgE** antibodies bound both chains with high specificity, and there was a good quantitative correlation between **IgE** antibody **binding** to Fel d I and both chain 1 ($r = 0.58$, $p < 0.01$) and chain 2 ($r = 0.47$, $p < 0.01$). Up to 70% of IgG or **IgE** anti-Fel d I antibodies could be absorbed by either chain 1 or chain 2, and both chains in combination produced similar absorption values in response to native Fel d I. Four mAbs were fully absorbed by chain 1, but not chain 2, and three mAbs were not absorbed by either chain. CONCLUSIONS: The results demonstrate a dichotomy between antibody **binding** to recombinant Fel d I chains, which may be explained by confirmational differences between the chains in the fluid phase or on solid supports. The results also suggest that chain 1 is an important site for mAb-defined **B-cell epitopes** on Fel d I.

L30 ANSWER 64 OF 77 CAPLUS COPYRIGHT 2002 ACS

1995:346835 Document No. 122:131033 Timothy grass pollen **allergen** Phl p II and a cDNA encoding it and T and **B cell epitopes**. Dolecek, Christiane; Vrtala, Susanne; Laffer, Sylvia; Steinberger, Peter; Kraft, Dietrich; Scheiner, Otto; Valenta, Rudolf (Biomay Produktions- und Handelsgesellschaft m.b.H., Austria). PCT Int. Appl. WO 9423035 A2 19941013, 25 pp. DESIGNATED STATES: W: AU, CA, FI, JP, NO, US; RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE. (German). CODEN: PIXXD2. APPLICATION: WO 1994-AT39 19940331. PRIORITY: AT 1993-672 19930401.

AB A cDNA encoding a peptide with the antigenicity of timothy grass pollen **allergens** Phl p II is cloned and characterized. The amino acid sequence is detd. from the cDNA and B and T cell epitopes of the mol. were identified algorithmically. The **allergen** cDNA is expressed in Escherichia coli and binds serum **IgE** of more than 60% of all those allergic to grass pollen and may therefore be used in the same way as the naturally occurring Phl p II for processes based on antigen-antibody interaction, mediator release and T cell reactivity.

L30 ANSWER 65 OF 77 CAPLUS COPYRIGHT 2002 ACS

1994:532227 Document No. 121:132227 T cell epitopes of ryegrass pollen **allergen**. Knox, Robert Bruce; Singh, Mohan Bir; Rolland, Jennifer; Blaher, Bella; Suphioglu, Cenk (University of Melbourne, Australia). PCT Int. Appl. WO 9404564 A1 19940303, 70 pp. DESIGNATED STATES: W: AT, AU, BB, BG, BR, BY, CA, CH, CZ, DE, DK, ES, FI, GB, HU, JP, KP, KR, KZ, LK, LU, MG, MN, MW, NL, NO, NZ, PL, PT, RO, RU, SD, SE, SK, UA, US, VN; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, DE, DK, ES, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1993-AU415 19930813. PRIORITY: US 1992-930060 19920814.

AB Peptides derived from Lol p V, a major protein **allergen** of Lolium perenne are described for use in the treatment and diagnosis of allergy. These peptides include at least one, preferably more, T cell epitopes of the Lol P V **allergen** and may be modified to improve their therapeutic properties and lessen side-effects from their use. Peptides derived from the sequence of an **allergen** cDNA and covering the complete sequence of the **allergen** were synthesized by automated Fmoc chem. and tested for human **IgE** and mouse/rabbit IgG epitopes by dot blotting. T-cells reacting to these epitopes were cloned by limiting diln. and cell lines established. Some of these epitopes were also recognized by B-cells.

L30 ANSWER 66 OF 77 MEDLINE

94365311 Document Number: 94365311. PubMed ID: 7521892. Monoclonal antibodies to group II Dermatophagoides spp. **allergens**: murine immune response, epitope analysis, and development of a two-site ELISA. Ovsyannikova I G; Vailes L D; Li Y; Heymann P W; Chapman M D. (UVA Asthma and Allergic Diseases Center, Charlottesville 22908.) JOURNAL OF ALLERGY AND CLINICAL IMMUNOLOGY, (1994 Sep) 94 (3 Pt 1) 537-46. Journal code: 1275002. ISSN: 0091-6749. Pub. country: United States. Language: English.

AB BACKGROUND: Group II **allergens** are a major cause of sensitization in patients allergic to mites. To facilitate the antigenic analysis of group II **allergens** and to develop improved methods of **allergen** detection, we compared IgG anti-group II antibody responses in inbred mouse strains and raised a panel of monoclonal antibodies (mAbs). METHODS: **IgE** antibody responses were compared by antigen-**binding** radioimmunoassay. Epitope specificity of the mAbs was analyzed by two-site **binding** assays and by cross-inhibition radioimmunoassays. RESULTS: Comparison of polyclonal IgG antibody responses in five BALB congenic strains showed that H-2d mice had poor responses, whereas H-2b and H-2k mice had strong, cross-reactive, IgG anti-group II responses. The specificities of nine anti-Der p II **IgE** mAbs raised in A/J mice were compared with specificities of seven mAbs produced previously. Most mAbs (11 of 16) recognized common epitopes on Der p II and Der f II: three were specific to Der p II, and two showed high **binding** to Der f II. Epitope analysis showed that the mAbs defined four cross-reactive, nonoverlapping sites on the group II **allergens**. **Binding** of several combinations of mAbs was compared, and a two-site ELISA for group II antigens was developed. Linear regression analysis showed an excellent correlation between results of this assay and group II radioimmunoassay of house dust samples ($n = 40$, $r = 0.85$, $p < 0.001$). CONCLUSIONS: There are multiple cross-reactive **B-cell epitopes** on group II **allergens**. The group II ELISA has several important applications, including assessment of environmental **allergen** exposure, monitoring of the efficacy of avoidance procedures, and standardization of commercial mite **allergen** extracts.

L30 ANSWER 67 OF 77 SCISEARCH COPYRIGHT 2002 ISI (R)

94:668452 The Genuine Article (R) Number: PM358. CLINICAL AND EXPERIMENTAL TRENDS IN HYPOSENSITIZATION. JAGER L (Reprint). FRIEDRICH SCHILLER UNIV, INST KLIN IMMUNOL, HUMBOLDTSTR 3, D-07740 JENA, GERMANY (Reprint). ALLERGOLOGIE (SEP 1994) Vol. 17, No. 9, pp. 400-403. ISSN: 0344-5062. Pub.

country: GERMANY. Language: German.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Hyposensitization has been developed starting from a wrong idea. Only during the last 15 years methods for an exact analysis of the underlying mechanisms became available - especially due to the progress in immunology. The first step was the characterization and purification of **allergens** from natural sources. The next one was the identification of **IgE-binding** epitopes. At present research activities are concentrated on T cell epitopes. By their means, above all, a modulation of the immune response could be achieved. This will be the scientifically-based way to the development of modified **allergen**.

L30 ANSWER 68 OF 77 MEDLINE

94311478 Document Number: 94311478. PubMed ID: 7518655. **IgE** and IgG cross-reactivity among Lol p I and Lol p II/III. Identification of the C-termini of Lol p I, II, and III as cross-reactive structures. van Ree R; van Leeuwen W A; van den Berg M; Weller H H; Aalberse R C. (Central Laboratory, The Netherlands Red Cross Blood Transfusion Service, University of Amsterdam, The Netherlands.) ALLERGY, (1994 Apr) 49 (4) 254-61. Journal code: 7804028. ISSN: 0105-4538. Pub. country: Denmark. Language: English.

AB In this study, the homologous C-termini of Lol p I, Lol p II, and Lol p III were shown to contain cross-reactive **B-cell epitopes**. This was demonstrated by inhibition studies with purified Lol p I, II, and III and synthetic peptides of their C-termini. It was ruled out that the observed cross-reactivity was caused by cross-contamination of the purified **allergens**. Both human **IgE** and IgG bound to the C-terminus of Lol p I. These antibodies were cross-reactive with Lol p II and, more specifically, with its C-terminus. Within a small panel of allergic patients, no cross-reactivity with Lol p III was found. A hyperimmune polyclonal rabbit antiserum against Lol p I also recognized the Lol p I C-terminus. As for human antibodies, cross-reactivity with Lol p II and its C-terminus was demonstrated. Cross-reactivity with Lol p III was demonstrated with C-terminal peptides, but not with native Lol p III. A polyclonal rabbit antiserum against Lol p II bound to the C-terminal peptides of both Lol p II and III. This **binding** was inhibited with Lol p I, confirming that cross-reactive structures exist not only on the C-termini of Lol p II and Lol p I, but also of Lol p III and Lol p I. The existence of cross-reactivity between Lol p I and Lol p II and III possibly contributes to the frequently observed cosensitization for these **allergens** in grass-pollen-allergic patients.

L30 ANSWER 69 OF 77 MEDLINE

95177937 Document Number: 95177937. PubMed ID: 7532944. **B-cell epitopes** of **allergens** determined by recombinant techniques; use for diagnosis and therapy of type I allergy. Valenta R; Vrtala S; Laffer S; Steinberger P; Ball T; Ferreira F; Scheiner O; Kraft D. (Universitat Wien, Austria.) ARBEITEN AUS DEM PAUL-EHRLICH-INSTITUT (BUNDESAMT FUR SERA UND IMPFSTOFFE) ZU FRANKFURT A.M., (1994) (87) 235-46. Journal code: 8912864. ISSN: 0936-8671. Pub. country: GERMANY: Germany, Federal Republic of. Language: English.

AB In the present manuscript, the immunological and functional in vitro properties of recombinant plant **allergens** are summarized. Recombinant tree pollen **allergens** (major birch pollen **allergen**-BetvI, birch profilin-BetvII) and recombinant timothy grass pollen **allergens** (PhlpI, PhlpV, and PhlpII) were compared with the natural counterparts regarding **IgE-binding** properties and capacity to release histamine from patients' basophils. In addition, experimental in vivo models of Type I allergy, based on recombinant **allergens**, are discussed. The major conclusion is that recombinant **allergens** can be seriously considered as

candidates for diagnosis of Type I allergy allowing to establish specific allergograms for the individual patients. The in vivo data obtained in mouse and primate systems indicate that recombinant **allergens** can be used to set up close-to-man models of Type I allergy. Such in vivo models are useful to test the effects of already established therapeutic approaches and also allow to develop therapeutical concepts which are based on the use of recombinant **allergens**. Examples of specific therapeutical concepts are presented.

L30 ANSWER 70 OF 77 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
1994:315554 Document No.: PREV199497328554. Epitope mapping of region 11-70 of ovalbumin (Gal d I) using five synthetic peptides. Elsayed, Said (1); Stavseng, Lene. (1) Allergiforskiningsgruppen, Haukelan Sykehus, N-5021 Bergen Norway. International Archives of Allergy and Immunology, (1994) Vol. 104, No. 1, pp. 65-71. ISSN: 1018-2438. Language: English.
AB Five successively located peptides, in region 11-70 of the major **allergen** of ovalbumin (OA) Gal d I (11-19, 0-33,34-46,47-55,56-70), were obtained by manual solid-phase peptide synthesis. These peptides to-ether with the previously reported OA region 1-10 comprise a segment of 70 amino acid residues located at the N-terminal of ovalbumin. The crude peptides were purified by gel filtration and reversed-phase high-performance liquid chromatographies and their sequences were verified. Polyclonal antibodies against the peptides conjugated to carrier protein (BSA) were raised in rabbits. Rocket line immunoelectrophoresis showed that four peptides (20-33,34-46, 47-55 and 56-70), could deflect OA-line immunoprecipitates. The peptide's affinity to rabbit polyclonal Ig was examined by quantitative precipitation inhibition and the results suggested that an epitope was encompassed in segments 34-55 and 47-55. Allergenicity was tested by inhibition of specific **IgE** **binding** of ovalbumin, using several sera and a serum pool from 16 egg-allergic patients. The results showed that the allergenicity was distributed over the whole region. These findings suggested that: (a) the region 11-70 of OA seemed not to encompass continuous epitopes. (b) the antigenicity of this region was convincing for peptides 34-46 and 47-55; (c) the allergenicity, though dependent on the patient serum used, was distributed over the whole of region 11-70; (d) peptide 11-19, although weak antigenically was capable of specific **IgE** inhibition; (e) human and rabbit polyclonal antibodies did not show analogous affinities to the present peptides

L30 ANSWER 71 OF 77 MEDLINE
94141068 Document Number: 94141068. PubMed ID: 7508462. Fine specificity of **B-cell epitopes** on Felis domesticus **allergen** I (Fel d I): effect of reduction and alkylation or deglycosylation on Fel d I structure and antibody **binding**. Vailes L D; Li Y; Bao Y; DeGroot H; Aalberse R C; Chapman M D. (Department of Medicine, University of Virginia, Charlottesville 22908.) JOURNAL OF ALLERGY AND CLINICAL IMMUNOLOGY, (1994 Jan) 93 (1 Pt 1) 22-33. Journal code: 1275002. ISSN: 0091-6749. Pub. country: United States. Language: English.
AB The repertoire of **B-cell epitopes** on the major cat **allergen**, Fel d I, was analyzed with monoclonal antibodies (MoAbs) in topographic mapping studies and in immunoassays with antigen derived from other cat (Felidae) species. Four essentially nonoverlapping epitopes on Fel d I, designated Fd1A to D, were defined by use of 15 anti Fel d I MoAbs in cross-inhibition radioimmunoassay. Only MoAbs directed against epitope Fd1B bound to putative Fel d I homologues in hair and dander extracts from seven other feline species (Panthera species, [n = 5], Leptailurus serval, and Leopardus pardalus). Quantitative monosaccharide analysis showed that Fel d I was a glycoprotein, containing high levels of fucose, as well as glucosamine, galactose, and mannose. **Binding** of MoAbs and human IgG or **IgE** antibody to native, reduced and alkylated or deglycosylated

Fel d I was compared by means of immunoprecipitation and immunoassay, and the effects of these treatments on the structure of Fel d I were analyzed by sodium dodecylsulfate-polyacrylamide gel electrophoresis. On reduction and alkylation, Fel d I dissociated into 14 kd and 3.2 kd peptides, and deglycosylation with trifluoromethane sulfonic acid produced a 12 to 14 kd peptide. These procedures resulted in a 100- to 1000-fold loss in murine or human antibody **binding** activity and caused significant loss of secondary structure, as judged by circular dichroism spectroscopy. Treatment with potassium hydroxide also caused a marked loss in antigenic reactivity. In contrast, enzymatic deglycosylation generated a 9 kd peptide, which showed strong reactivity with murine and human antibodies, comparable to native Fel d I. The results show that MoAbs define a broad repertoire of **B-cell epitopes** on Fel d I, one of which is expressed by other cat species. These epitopes are conformational and do not appear to involve oligosaccharide residues.

L30 ANSWER 72 OF 77 CAPLUS COPYRIGHT 2002 ACS
1994:97295 Document No. 120:97295 Several epitope structures of Chi T I component III. II. Construction, expression and purification of fusion proteins that carry an allergenic sequence functioning as T- and **B -cell epitope**. Rihs, H. P.; Rozynek, P.; Jans, D. A.; Hankeln, T.; Baur, X. (BGFA, Bochum, W-4630, Germany). Mol. Biol. Immunol. Allergens, 297-8. Editor(s): Kraft, Dietrich; Sehon, Alec H. CRC: Boca Raton, Fla. (English) 1993. CODEN: 59QMA6.
AB .beta.-Galactosidase-**allergen** epitope fusion proteins were prepd. in Escherichia coli by std. cloning methodol. and fusion proteins contg. the Chi t I component III epitope (peptide 1-17) were purified by affinity chromatog. Four plasmid systems were obtained, pRI1 and pRI2 encoding epitope 1-17 once and in tandem, resp,. followed by DNA encoding .beta.-galactosidase residues 6-1023, and pB1B2 encoding epitope 1-17 and the streptococcal human serum albumin **binding** domain and pRIT24 encoding epitome 1-17 double fusion proteins with a streptococcal HSA **binding** domain and staphylococcal IgG **binding** domain. The four systems will be used to measure the **IgE binding** capacity of the expressed fusion proteins.

L30 ANSWER 73 OF 77 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
1993:269968 Document No.: PREV199396000193. Alpha-amylase of Aspergillus oryzae (Asp o I) as **allergen** in bakers' disease. Sander, Ingrid (1); Baur, X.; Isringhausen-Bley, S.; Rozynek, P.. (1) Gilsingstrasse 14, W-4630 Bochum 1 Germany. Allergologie, (1993) Vol. 16, No. 3, pp. 87-90. ISSN: 0344-5062. Language: German. Summary Language: German; English.
AB Not only natural components of flour but also baking additives are associated with a high risk of developing allergic respiratory diseases. In a collective of 89 bakers 16% showed a sensitization to the starch hydrolyzing enzyme alpha-amylase of Aspergillus oryzae indicated by significant levels of specific **IgE** antibodies (EAST). With electrophoretic separation of the commercial alpha-amylase preparation, immunoblot and enzyme detection by iodine-starch visualization we could identify the alpha-amylase itself as the major allergenic component. We named it "Asp o I" according to IUIS nomenclature. The primary structure and X-ray crystallography of this enzyme are well known and were used for molecule modeling on a silicon graphics computer with the program Insight II (Biosym). The position of the four disulfide bridges, the Ca-2+ **binding** sites and the glycosilation site are indicated. Furthermore we analyzed the position of potential **B-cell epitopes** using the algorithms of Jameson and Wolf (9).

L30 ANSWER 74 OF 77 SCISEARCH COPYRIGHT 2002 ISI (R)
91:507056 The Genuine Article (R) Number: GD777. IMMUNOLOGICAL CROSS-REACTIVITY OF HEMOGLOBINS IN THE DIPTERA FAMILY CHIRONOMIDAE. BAUR X (Reprint); LIEBERS V; MAZUR G; BECKER W M; KAGEN S L; KAWAI K. RUHR UNIV BOCHUM, BERUFSGENOSSENSCHAFTL FORSCHUNGSINST ARBEITSMED, GILSINGSTR 14,

W-4630 BOCHUM 1, GERMANY (Reprint); MED COLL WISCONSIN, APPLETON, WI, 00000; FORSCHUNGSINST BORSTEL, W-2061 BORSTEL, GERMANY; UNIV MUNICH, KLINIKUM GROSSHADERN, MED CLIN 1, DEPT PNEUMOL, W-8000 MUNICH 70, GERMANY; TOYAMA MED & PHARMACEUT UNIV, FAC MED, DEPT BACTERIOL & IMMUNOL, SUGITANI, TOYAMA 93001, JAPAN. ALLERGY (1991) Vol. 46, No. 6, pp. 445-451. Pub. country: GERMANY; USA; JAPAN. Language: ENGLISH.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Polyclonal human **IgE** antibodies of patients sensitized to one species of the Diptera family Chironomidae as well as polyclonal rabbit anti-Chi t I hyperimmune serum recognize antigenic sites present in nearly all of the 33 species of this insect family. Evolutionary distantly related genera usually show weaker antibody **binding**. According to the pattern of reactivity of rabbit anti-Chi t I-component III, expression of the epitopes of this molecule varies considerably in the genera Chironomus and Glyptotendipes; it appears to be almost totally absent in all other species. Of five monoclonal antibodies raised against Chi t I-component III, two recognize an epitope which is expressed in nearly all closely related species. Three monoclonal antibodies recognize epitopes which are expressed in only a few species of the same genus. Our results demonstrate the presence of common as well as species-specific epitopes in chironomid hemoglobins which behave as potent inhalant **allergens**.

L30 ANSWER 75 OF 77 CAPLUS COPYRIGHT 2002 ACS
1992:589575 Document No. 117:189575 Epitopes on **allergens**. Jager, L.; Diener, C.; Mueller, W. D.; Schlenvoigt, G. (Dep. Clin. Immunol., Friedrich Schiller Univ., Jena, O-6900, Germany). New Trends Allergy III, [Int. Symp.], 3rd, Meeting Date 1990, 33-47. Editor(s): Ring, Johannes; Przybilla, Bernhard. Springer: Berlin, Germany. (English) 1991. CODEN: 58CHAG.

AB A review with 58 refs. By conventional immunol. and physicochem. techniques, natural **allergens** have been sepd. into antigenic and allergenic fractions. During the last years new methodol. developments enabled investigation of their structures in detail. The first step was the identification of antigenic and allergenic (**IgE-binding**) epitopes on these mols. This has been done for several major **allergens** from pollen, insect, fungal, and epidermal **allergens**. Usually, 2-6 antigenic and 1-3 allergenic sites have been found. In some cases, the primary structure of such epitopes or even the complete **allergen** has been uncovered. In a few of them, (Der p I, Chi t I e.g.) even the three-dimensional structure could be disclosed. Obviously sequential epitopes dominate, at least in grass pollen **allergens**. The methods which have been applied are monoclonal antibodies, improved techniques of peptide anal. and synthesis, and cloning procedures. These developments are very important both from practical and theor. points of view. They will improve diagnostic procedures and disclose th basis of often surprising cross-reactivities. More important is the fact that **B-cell epitopes** (being active during the manifestation of the allergic reaction) are obviously not identical with T-cell epitopes (important during sensitization). This fact could open new ways for a scientific-based approach to immunotherapy. Other prospective developments concern the presentation of these epitopes together with MHC structures, the identification of possibly individual-specific patterns of sensitization, and a reevaluation of the exact mechanisms of the initial step of **IgE**-mediated reactions. The dogma that bridging of membrane-bound **IgE** antibodies is induced by identical epitopes obviously has to be modified.

L30 ANSWER 76 OF 77 MEDLINE
90154809 Document Number: 90154809. PubMed ID: 1689351. Conformational stability of **B cell epitopes** on group I and group II Dermatophagoides spp. **allergens**. Effect of thermal and

chemical denaturation on the **binding** of murine IgG and human **IgE** antibodies. Lombardero M; Heymann P W; Platts-Mills T A; Fox J W; Chapman M D. (Department of Medicine, University of Virginia, Charlottesville 22908.) JOURNAL OF IMMUNOLOGY, (1990 Feb 15) 144 (4) 1353-60. Journal code: 2985117R. ISSN: 0022-1767. Pub. country: United States. Language: English.

AB The conformational stability of **B cell epitopes** on the 25-kDa group I and 14-kDa group II mite **allergens** was compared by using heat-treated or chemically denatured **allergens** to inhibit the **binding** of native 125I **allergens** to murine mAb or to human **IgE** antibodies. Structural changes after treatment were assessed by SDS-PAGE and circular dichroism spectroscopy. Heating for 1 h at greater than 75 degrees C, treatment at pH 2.0 or pH 12.0, or with 6M guanidine or 6M urea, reduced the **binding** of the group I **allergens** to mAb or **IgE** antibodies by 10- to 1000-fold. The group II **allergens** were heat stable and even after prolonged heat treatment (5 h at 75 degrees C or 30 min at 100 degrees C) their antibody **binding** activity was reduced by less than twofold. The group II **allergens** were also resistant to pH and to denaturation with 6M guanidine. However, after reduction and alkylation, antibody **binding** sites on both the group I and group II **allergens** were destroyed. Reduction of disulfide bonds with 2-ME caused a marked shift in the molecular mass of group I **allergens** on SDS-PAGE, from 25 kDa to 28-31 kDa. Reduction and alkylation also generated two high m.w. forms of Der p I and Der f I. After heating (100 degrees for 30 min), both Der f I and Der f II retained significant secondary structure, as judged by circular dichroism spectroscopy, but on reduction they showed the typical spectra of fully denatured proteins (greater than 85% random structure). The results show clear differences between the susceptibility of **B cell epitopes** on the group I and group II **allergens** to denaturation. Despite these differences in stability, both **allergens** are equally potent immunogens for **IgE** antibody responses in man. The results support the view that the physical properties of **allergens** (low m.w. and solubility), limiting low dose exposure (1 to 10 ng/day), and host genetic and immunoregulatory processes, are more important than gross structural features in the induction and maintenance of **IgE** antibody responses.

L30 ANSWER 77 OF 77 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
1997:296284 Document No.: PREV199799595487. Extraordinary stability of **IgE-binding** Parietaria pollen **allergens** in relation to chemically bound flavonoids. Gonzalez Romano, M. L.; Gallego, M. T.; Berrens, L. (1). (1) Res. Lab. C.B.F. LETI, Calle del Sol, S. Poligono Ind. Norte, 28706 Tres Cantos, Madrid Spain. Molecular Immunology, Vol. 33, No. 17-18, pp. 1287-1293. ISSN: 0161-5890. Language: English.

AB It is known that the skin-active and **IgE-binding** components in Parietaria pollen extracts are not restricted to the predominant protein **allergens** of M-r 12 000-15 000, but are present as well among the naturally occurring constituents of M-r lt 10 000. Indeed, the **IgE-binding** Parietaria pollen components are quite heterogeneous, ranging from high- to low-molecular mass, whereby the **IgE-binding** epitopes display an unusual chemical stability. Furthermore, the pollen of Parietaria species demonstrably contain a high proportion of flavonoid pigments. Since these pollen grains cannot be collected entirely free from non-pollen plant parts, the usual allergenic extracts of Parietaria encompass both the polyphenolic substrate molecules and the enzyme polyphenoloxidase as ingredients for the oxidative generation of flavonol-protein conjugates during the extraction process. In the present work this is illustrated by spectroscopic analyses of the free and bound flavonoids in Parietaria

pollen extracts, as well as of the peptide fragments produced from the allergenic proteins by enzymatic or chemical hydrolysis. None of these relatively harsh treatments had a significant effect on the **IgE-binding** properties of the allergenic (sub-)components, even though detectable proteins in isoelectric focusing and immunoblotting were lost. It is proposed that the extraordinary stability of **IgE-binding** Parietaria components over a wide molecular range may be attributed to chromophoric flavonoid side-chains as (parts on the corresponding **B-cell epitopes**.

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